Cellometer® Vision

User Manual

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Technical Support:
Nexcelom Technical Support is available to assist with any technical issues or questions you might have during the installation or operation of the Cellometer Vision:

Email: support@nexcelom.com
Phone: +1-978-327-5340
Fax: +1-978-327-5341

Technical Support is available: Monday – Friday 8:00 AM to 5:00 PM Eastern US Time.

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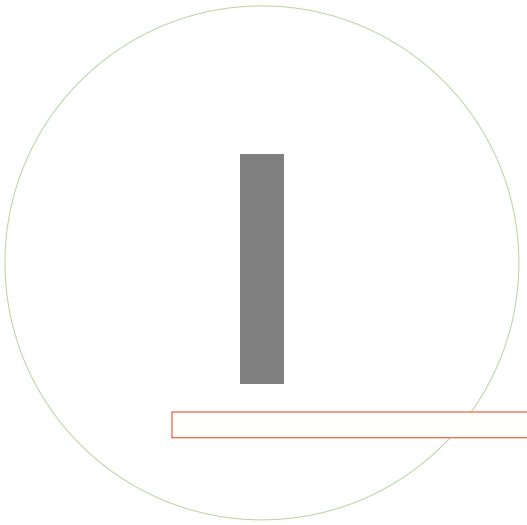

Introduction

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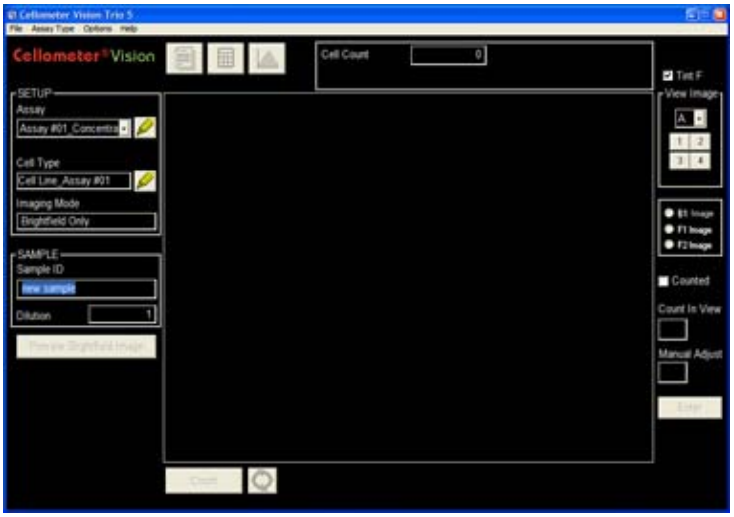
What is Cellometer Vision

Cellometer® Vision is a compact, automated cell analysis system that can count cells, measure cell sizes, and detect fluorescence properties of cells. The basic principle of the Cellometer automatic cell counter is imaging cytometry. Cells are loaded into the Disposable Counting Chamber and automatically spread into a thin layer by capillary action. Cellometer Vision then captures images of cells in the counting chamber, analyzes the number of cells, sizes and fluorescence intensity of each cell, then converts this data into concentration, size and fluorescence histograms and scatter plots.

The Cellometer Vision system consists of 3 main components:

1. Cellometer Vision instrument:

2. Cellometer Vision Software and Controller Laptop (Included with instrument and pre-loaded with software)



3. Disposable Counting Chambers

- Each disposable counting chamber accommodates 2 individual samples and can be loaded through either port.
- Simply pipette 20µL into one of the ports with any standard single channel pipette.
- Cellometer Vision comes with a starter set of 25 slides. Slides can be ordered directly from Nexcelom or your authorized Nexcelom dealer.

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Generating Data with Cellometer Vision

Generating cell sample information is quick and easy. Once the instrument is setup for your particular assay, only 3 basic steps are needed to get results:

1. prepare sample and load counting chamber

2. Insert counting chamber into instrument

3. Use Software to acquire images, analyze samples, and view results

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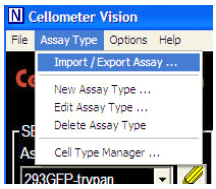
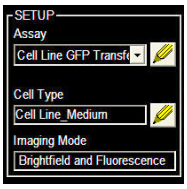
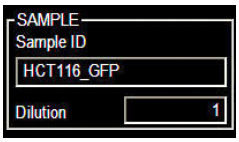
Quick Operation Instructions

Below are the basic steps that are followed to run any assay:

Step	Instructions:	
1.	**Setup or Select an Assay.** (a) Import or Setup a new assay from the Assay Type drop down menu, (b) Select an existing assay from the drop down list or (c) edit an existing assay.	 **a. and c.**  **b.**
2.	**Input Sample ID and Dilution Factor.**	
3.	**Prepare sample, load disposable counting chamber and insert into instrument.**	
4.	**Click 'Preview Brightfield Image' and ensure image is in focus.**	
5.	**Adjust Focus** Slowly turn the black focus wheel until optimal cell counting focus is achieved. *Live cells and training beads will have a bright center and a clear defined edge.*	
6.	**Click 'Preview F1 Image' and adjust exposure time if necessary.** If using Cellometer Vision Trio in dual fluorescence mode: click '**Preview F2 Image**' and adjust exposure time if necessary	
7.	**Click 'Count' to begin counting process.**	

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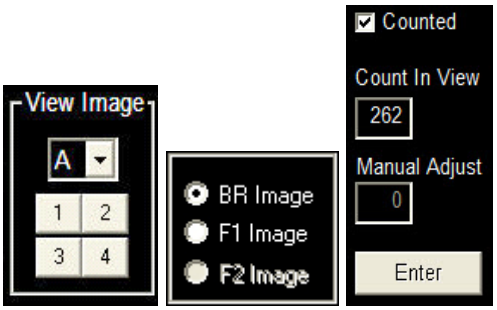
8.	**Review Counting Results and generate desired reports.** Counting results will automatically display on-screen. Select desired reports/graphs to display, export/print data, or click Done to clear window and review on-screen images.	
9.	**Navigate and review images on screen:** (a) Select a section of the image to view, (b) toggle between the brightfield (BR) and fluorescence (F1 or F2) images, or (c) manually adjust counted cells	 **a.** **b.** **c.**
10.	If desired, click (a) Re-display Counting Results, (b) Launch Sample Adjustment Calculator, or (c) Display Size Distribution	 **a.** **b.** **c.**

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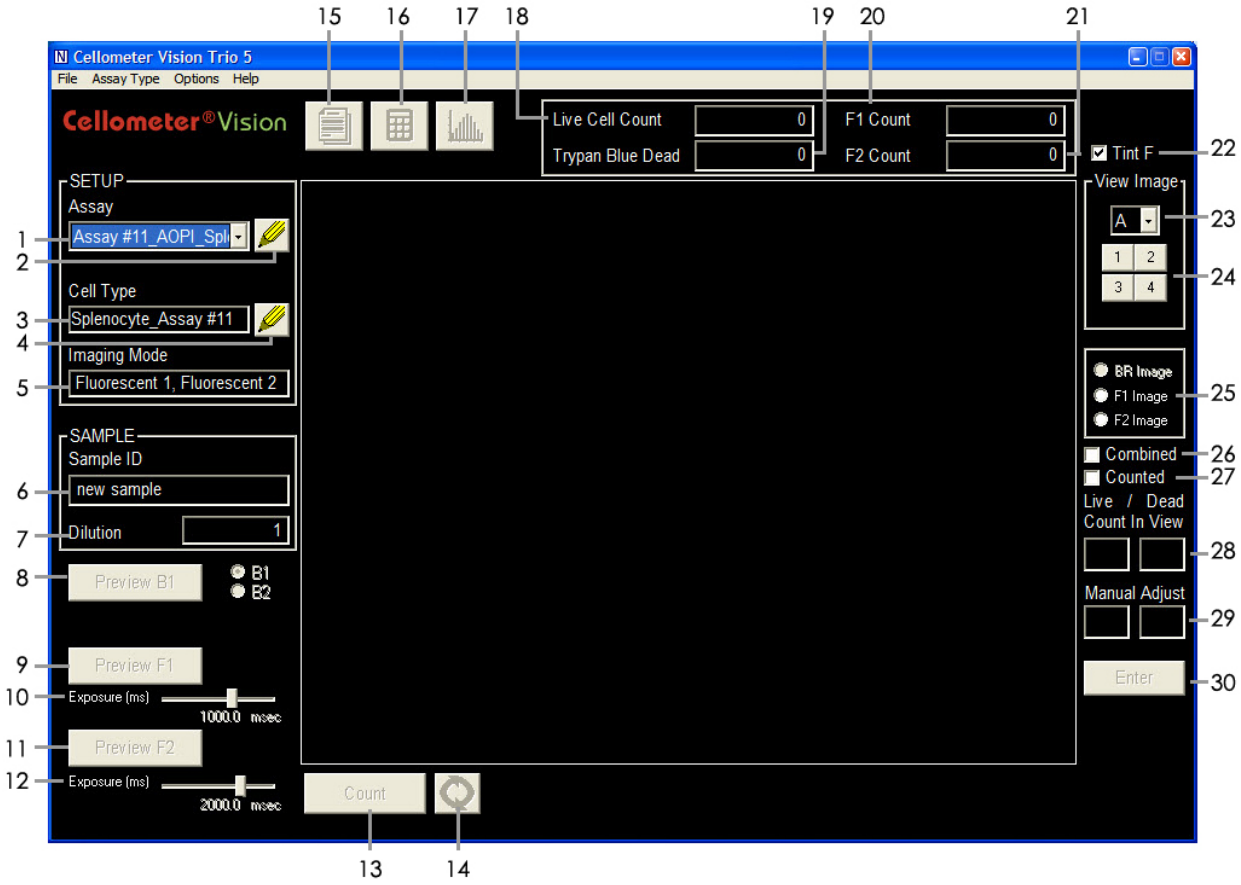
Software Overview



Item:	Name:	What it does:
1	**Select Assay**	Selects a saved assay from the Assay Library
2	**Modify Assay**	Modifies parameters of currently selected Assay
3	**Cell Type Display**	Displays the selected cell type
4	**Modify Cell Type**	Modifies parameters of currently selected Cell Type
5	**Imaging Mode Display**	Displays current imaging mode settings
6	**Sample ID Input**	Allows user to input name/ID/# of current sample
7	**Dilution Factor Input**	Allows user to input dilution factor
8	**Preview Brightfield Image**	Previews brightfield image before counting
9	**Preview F1 Image**	Previews the fluorescent image: F1
10	**F1 Exposure Adjustment**	Adjusts exposure time for F1 image (in milliseconds)
11	**Preview F2 Image**	Previews the fluorescent image: F2
12	**F2 Exposure Adjustment**	Adjusts exposure time for F2 image (in milliseconds)
13	**Count (or Speed Count) Button**	Initiates counting procedure
14	**Recount Button**	Recounts currently loaded image
15	**Display Counting Results**	Opens counting results box
16	**Sample Adjustment Calculator**	Launches Sample Adjustment Calculator
17	**Display Size Distirbution**	Opens size distribution histograms
18	**Live Cell Count Display**	Shows number of live cells in current view
19	**Trypan Blue Dead Display**	Shows number of Trypan Blue stained (dead) cells in current view
20	**F1 Counted**	Shows number of fluorescent positive cells in F1 image

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21	**F2 Counted**	Shows number of fluorescent positive cells in F2 image
22	**Tint F**	Check to display fluorescent cells in false color
23	**Captured Image Selection**	Toggles view between A, B, C, and D captured images
24	**Captured Image Quadrant Buttons**	Toggles view between quadrant 1, 2, 3 and 4 of each captured image
25	**Imaging Mode View Selection**	Toggles view between Brightfield (BR), Fluorescence 1(F1) and Fluorescence 2 (F2)
26	**Combine**	Displays an overlay of F1 and F2 images
27	**Counted**	Displays counted images
28	**Live/Dead Count in View**	Displays total number of live or dead counted cells in view
29	**Manual Adjust**	Allows user to manually adjust counting results.
30	**Enter**	Updates counting results after entering Manual Adjust values

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Terms and Icons Used

Assay	A set of configuration parameters that include the instrument settings, i.e. fluorescence modes, exposure times, counting method and associated cell types.
Cell Type Parameters	A set of configuration parameters specific to a cell used in an assay such as cell size, fluorescence properties and decluster properties
Decluster	A software function used to distinguish and individually count cells from a cluster of cells
Brightfield	A standard imaging mode of the instrument using brightfield optics for viewing and counting total cells, measuring cell sizes and determining viability using trypan blue
Fluorescence	The fluorescence imaging capabilities and modes of the instrument. Used to detect fluorescence properties of cells. Vision Duo has one fluorescence channel, while Vision Trio has two.
Brightfield Image	Image displayed on screen after it has been acquired from the brightifeld optics.
Fluorescent Image	Image displayed on screen after it has been acquired from the fluorescence optics.
F1, F2	F1 is the first fluorescence image. F2 is the second fluorescence image.
Threshold	The limit to include or exclude a cell in a particular category (counted, not counted, live/dead, fluorescence positive/negative, etc.) based on a particular property of the cell (i.e. size, fluorescence level, bright center, etc)
Count	The command to initiate an image capture and analysis of a sample
Speed Count	The command to initiate an image capture and analysis of a sample in Speed Count mode
	Modify Assay or Cell Type parameters: click to quickly access parameters from the main screen.
	Generate Data and Reports: click to view Counting Results box after initial pop-up screen has been cleared.
	Sample Adjustment Calculator: click to display the Sample Adjustment Calculator. Useful for sample adjustment to get desired concentration or total cell number.
	View Size Distribution: click to display size distribution after the initial pop up box has been cleared.
	Recount: click to recount the same acquired image after Assay or Cell parameters have been modified.
	Green Circle: Indicates a counted cell. In brightfield mode, indicates a live cell when running Trypan Blue assays. In fluorescent mode, indicates a fluorescence positive cell.
	Red Circle: In brightfield mode, indicates a dead cell (Trypan Blue Positive) when running Trypan Blue assays. In fluorescent mode, indicates a fluorescence negative cell.

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Getting Started

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Cellometer Vision System Components

The Cellometer Vision comes with the following components:

- Cellometer Vision Instrument
- Laptop Controller (and associated accessories and documentation)
- USB 2.0 Cable
- Cellometer Vision Power Supply
- Cellometer Vision Software CD
- User's Manual
- Consumable Starter Pack (Disposable Counting Chamber Slides and Training Bead Solution)

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FRONT/BACK VIEW

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Setting up Cellometer Vision

All Nexcelom products undergo a rigorous quality inspection prior to shipment and all reasonable precautions are taken in preparing them for shipment to assure safe delivery.

The instrument should be unpacked and inspected for mechanical damage upon receipt. Mechanical inspection involves checking for signs of physical damage such as: broken knobs, scratches, dents, etc.

If damage is apparent, or any components are missing, please immediately contact Nexcelom (+1-978-327-5340 or support@nexcelom.com) or your local dealer.

After unpacking the instrument and laptop, plug the Cellometer Power Cable into the back of the instrument, and connect the instrument to the laptop using the enclosed USB 2.0 cable. **If you use a computer that is not supplied directly from Nexcelom Bioscience (or Authorized Distributor), the software must be installed BEFORE the USB 2.0 cable is connected.** If you are using a computer supplied directly by Nexcelom Bioscience (or authorized distributor), connect the instrument to the computer with the enclosed USB 2.0 cable.

The laptop is pre-configured with Cellometer Vision software and Microsoft Excel (North America only). No additional setup or configuration is required. If software re-installation is required, please contact Nexcelom Technical Support or your local dealer.

Connecting Cellometer Vision to Laptop

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Initial Configuration: Setting Background Image

Before using Cellometer Vision, a background image (image of the system itself without a counting chamber) must be obtained to normalize the instrument. Unless the instrument is moved, there is generally no need to take a background image again.

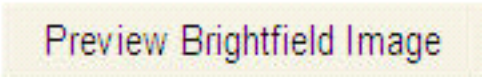
Step 1. Ensure Cellometer Vision is connected to the laptop controller and turn on both the Cellometer and the Laptop

Step 2. Double Click on the Cellometer Vision icon on the Laptop desktop to launch the software

Step 3. The first time the software launches, you may see this message. Click '**OK'** to continue. **NOTE:** You will not see this message when using a laptop supplied directly by Nexcelom Bioscience (or Authorized Distributor).

Cellometer - Error
Can not load background image specified as "C:\Program Files\Nexcelom\Background.bmp"
Please take a background image after the program starts.
OK

Step 4. Click '**Preview Brightfield Image'** and verify a gray uniform background image.



Step 5. From the Menu bar, select **'Options' > 'Take Background image'** to begin image acquisition.

NOTE: This procedure can take several seconds since it automatically adjusts the exposure to obtain the optimal background image.

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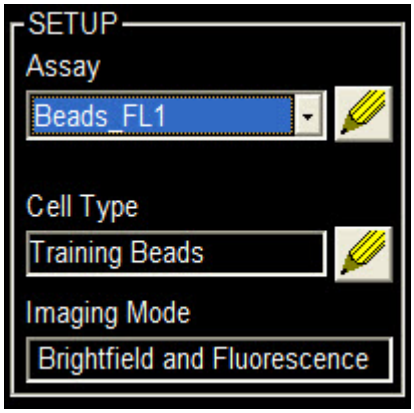
Using Vision for the First Time

Before using Cellometer Vision for running samples, use the training bead solution to ensure the instrument is setup and configured properly.

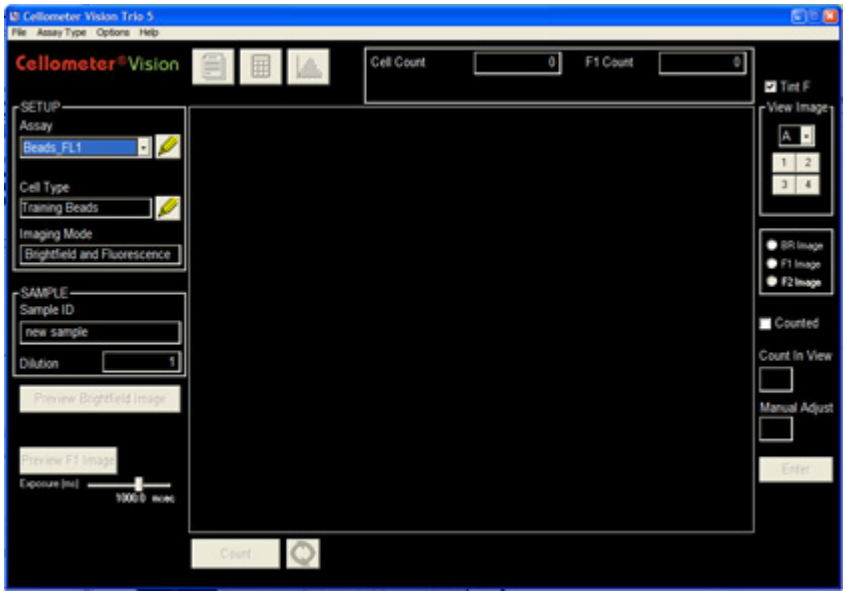
Step 1.

- Vortex Training Bead Solution at low speed for 10 seconds
- Remove a clean Disposable Counting Chamber and place on a clean Kimwipe
- Pipette all 20µL of solution into one side of the chamber. Capillary force automatically spreads the sample within the counting chamber.
- Hold the loaded chamber in the white area (taking care to stay away from the clear optical window area). Insert chamber into slot in the front of the instrument.

Step 2. Select "Training Beads" from the Assay type drop down menu



Step 3. Click '**Preview Brightfield Image'**. You should see an image similar to the one below:



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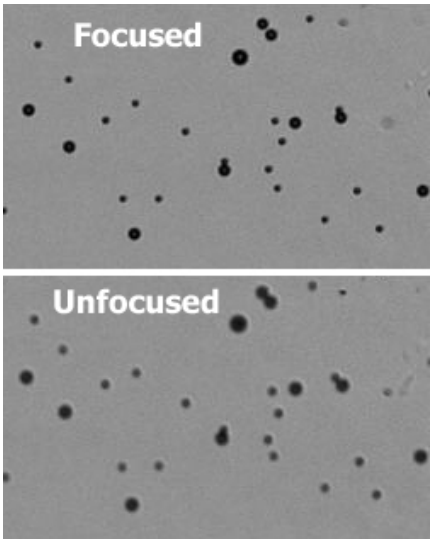
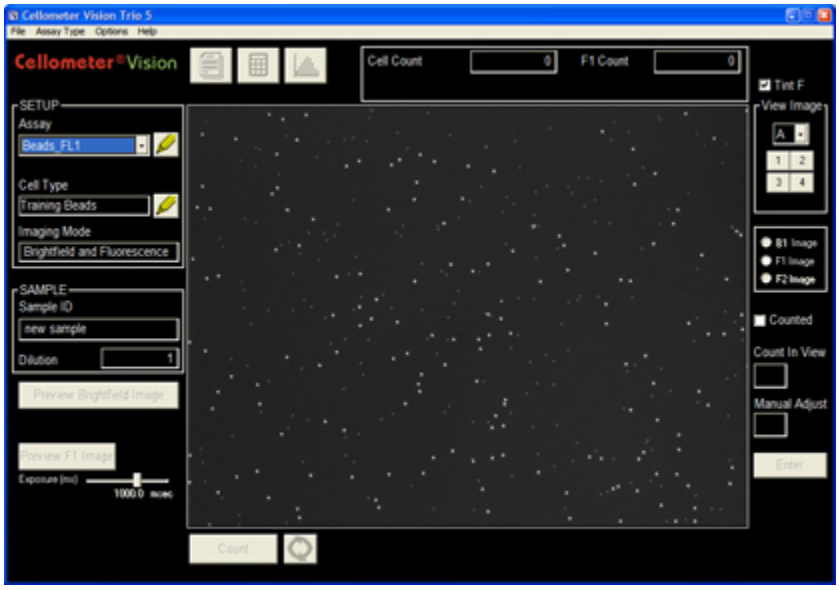
Step 4. Slowly turn the black focus wheel until optimal cell counting focus is achieved:
(NOTE: Live cells and training beads will have a bright center and a clear defined edge)



Step 5. Click '**Preview F1 Image**'. You should see an image like the one below:



Step 6. Click Count (or Speed Count) to begin the counting process.

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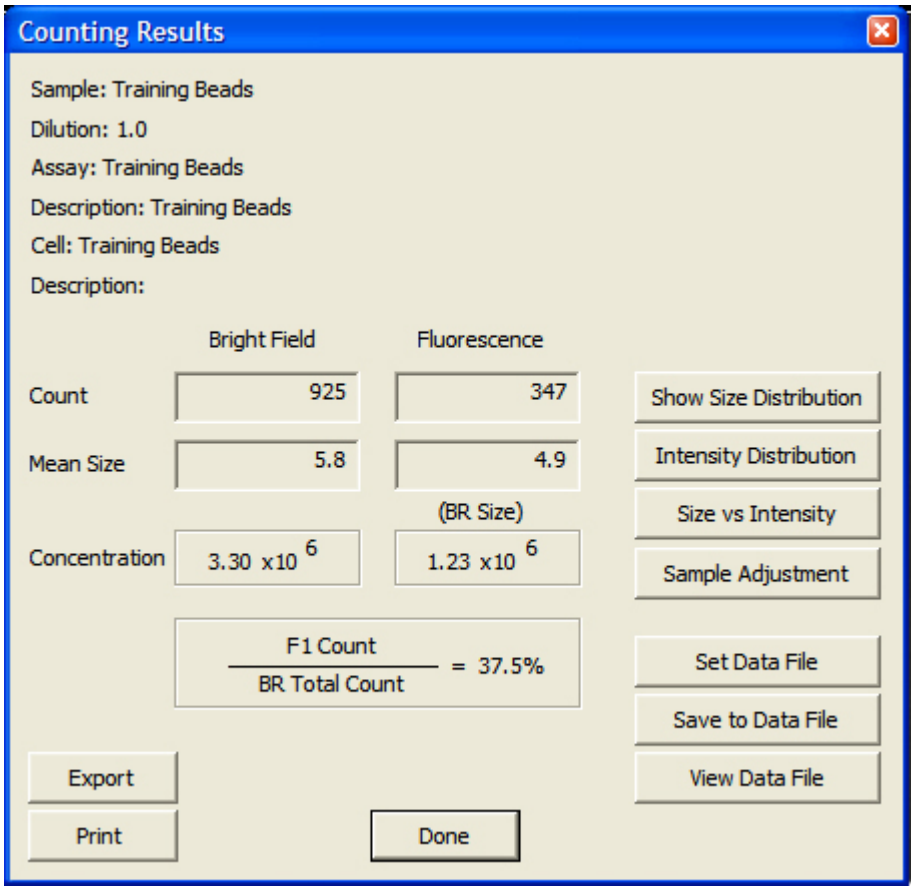
Step 7. The counting results box will display upon conclusion of the counting process. You should see cell counts in both the brightfield and fluorescence boxes. **The results should be approximately what is pictured below. If the these figures are grossly out of range, please contact Nexcelom support at +1-978-327-5340 or support@nexcelom.com, or your authorized distributor.**



Initial setup and configuration are now complete. You are ready to start using Cellometer Vision. Section III contains tutorials of common applications to help familiarize yourself with the instrument and its functions.

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Tutorials

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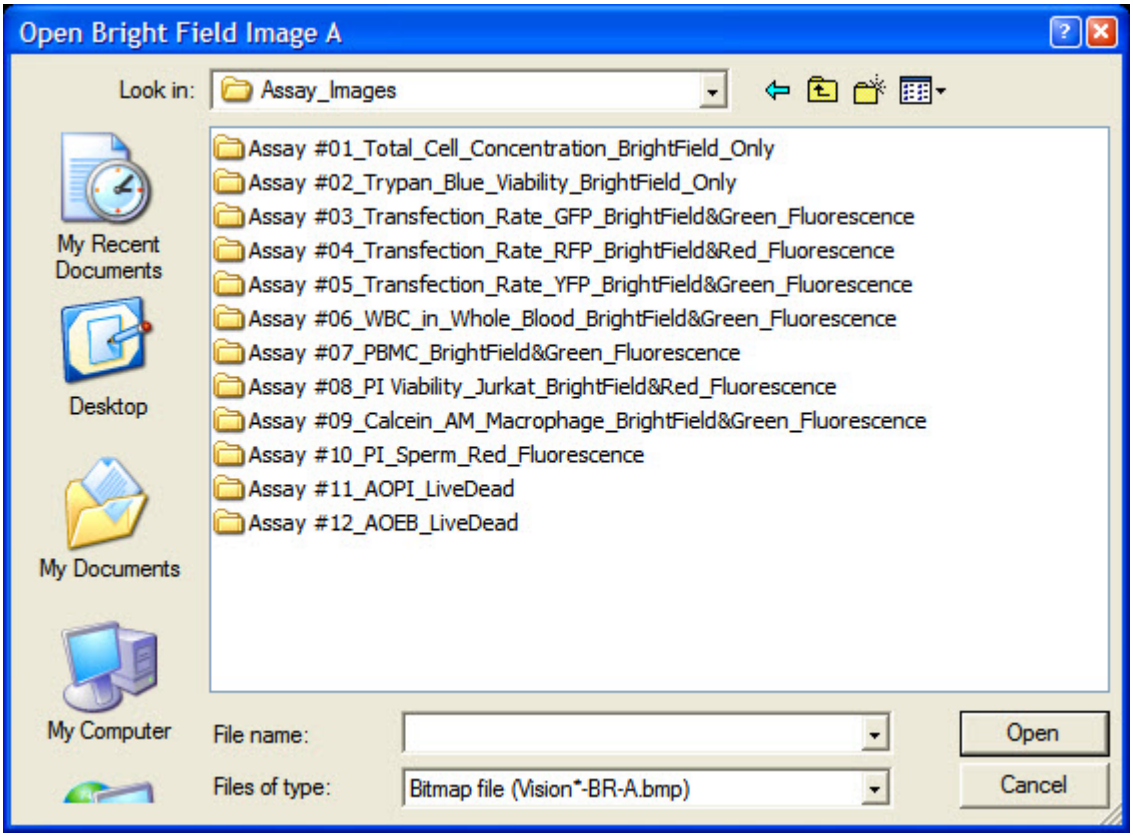
Tutorial Overview

The following tutorials are intended as a guide to performing various cell-based assays using the Vision. General sample preparation hints are included for each tutorial as well as instrument and software operation instructions. Each of the assays can also be performed using the sample images included in the software as a demonstration of instrument and software operation. Sample images for each assay can be found at: C:\Program Files\Nexcelom_Vision\Assay_Images



Note: Assay parameters for assays that are included in the software are locked and can not be edited. To customize any assay to suit your particular application, please see **Modify Default Assay Parameters** for instructions on how to modify and save as a new Assay.

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Assay #01 Total Cell Concentration Measurement

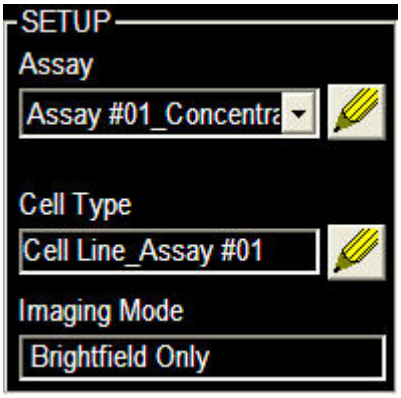
After taking brightfield images of a cell sample, all cells are counted to determine total cell concentration.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

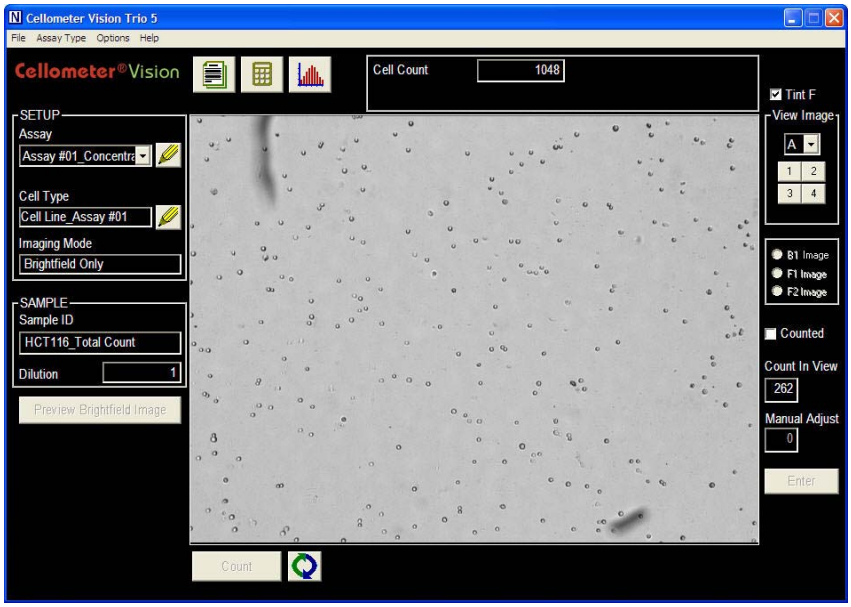
Step 1. Prepare cells suspended in growth media or PBS

Step 2. Load 20µL of cell sample in one side of the cell counting chamber and insert into instrument

Step 3. Select **Assay #01_Concentration** from **Assay SETUP** drop-down menu:



Step 4. Click **'Preview Brightfield image'**:



Step 5. Adjust focus if necessary

Step 6. Click **'Count'**

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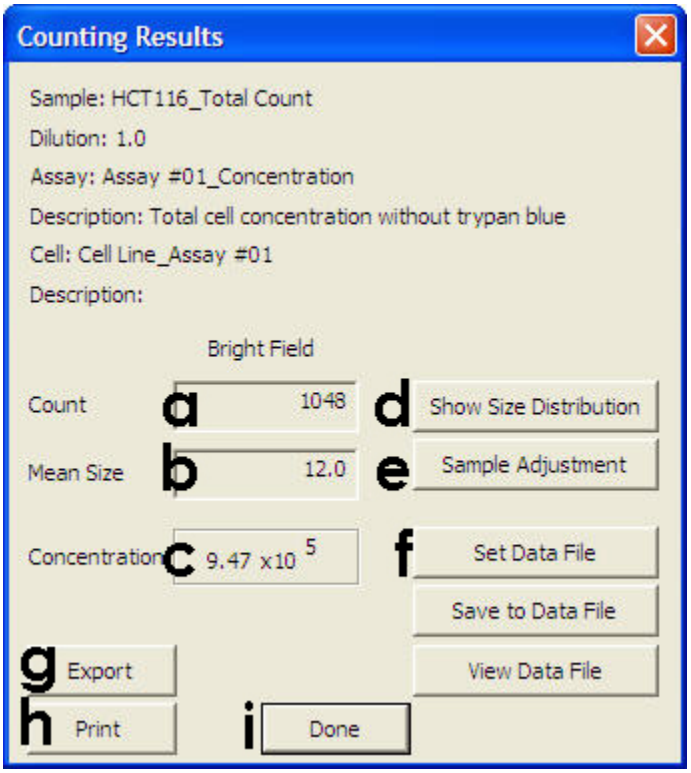
Step 7. Review Counting Results and display data as needed:



a. Count: *total number of cells counted*
b. Mean Size: *mean diameter of the counted cells*
c. Concentration: *cell concentration (/mL)*
d. Show Size Distribution: *displays cell size histogram generated using brightfield image*
e. Sample Adjustment: *launch sample adjustment calculator*
f. Save to Data File: *record counting results to a data file*
g. Export: *stores all raw data, assay parameters and graphs in an Excel file*
h. Print: *send current counting results to printer*
i. Done: *close counting results window*
Note: After clicking **'Done'** *the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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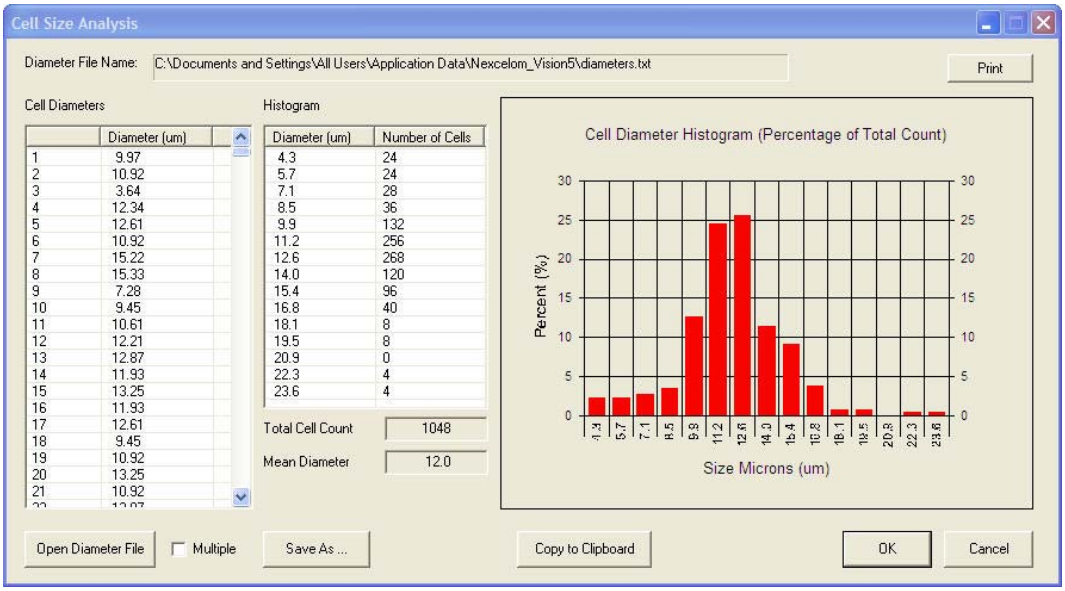
(d) Show Size Distribution:



Print: *print cell size histogram*
Save As: *save cell diameter values to a data file*
Open Diameter File: *open an existing cell diameter file*
Multiple: *check to load and display multiple cell diameter files (up to 5)*
Copy to Clipboard: *copy diameter data and histogram to Clipboard. This data can be pasted into a Word, Excel or PowerPoint document*
OK
Cancel

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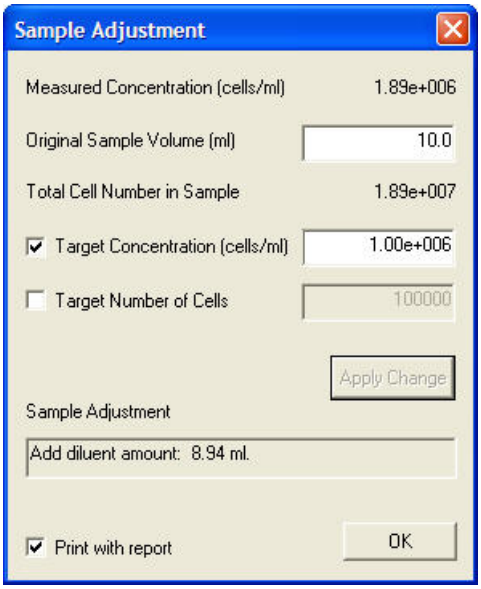
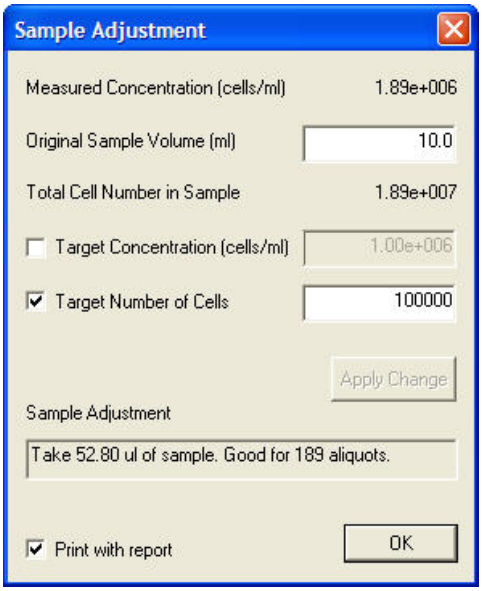
(e) Sample Adjustment:

***Measured Concentration**: cell concentration measured by Cellometer*
***Original Sample Volume**: total volume of cell sample*
***Total Cell Number in Sample**: total number of cells in the original sample*
***Sample Adjustment**: instructions for sample adjustment to obtain desired results*



Calculator #1: adjust to obtain target cell concentration
***Target Concentration**: desired cell concentration (/mL)*
***Apply Change**: to calculate adjustment*



Calculator #2: adjust for target number of cells
***Target Number of Cells**: desired number of cells*

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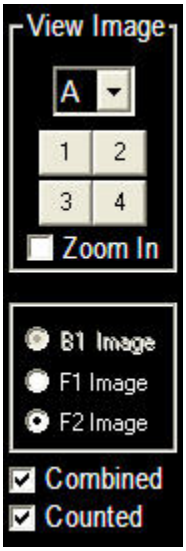
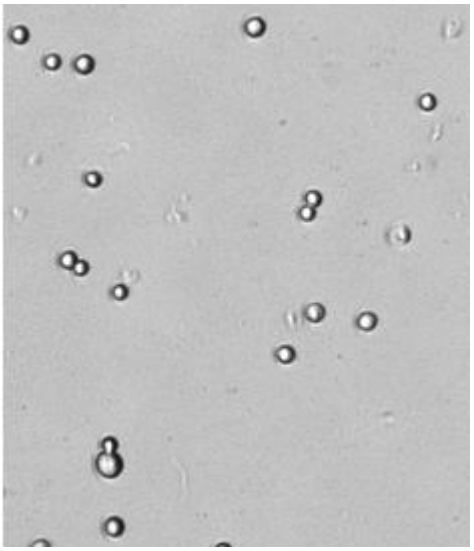
Step 8. Review Images on-screen

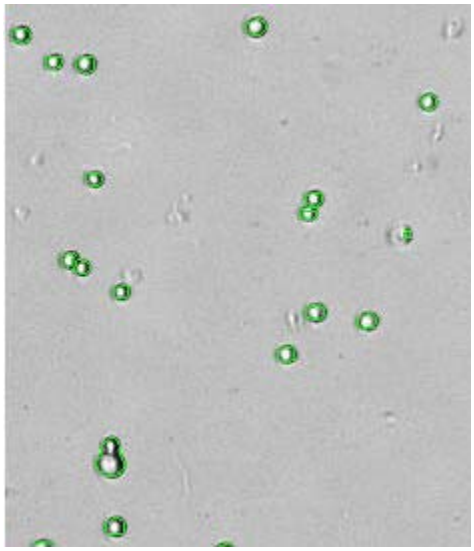


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Cell Image before counting



B. Counted cell image. Green outline indicate counted cells

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Assay #02 Trypan Blue Viability

Trypan blue is routinely used to determine cell viability. Trypan blue penetrates and stains dead cells and leaves live cells unstained. After taking brightfield images of the stained sample, live and dead cells are counted to determine total, live and dead cell concentrations as well as compute percent viability.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Mix cell sample with trypan blue and incubate using the same method as when manually counting

Step 2. Load 20µL of cell sample in one side of the cell counting chamber and insert into instrument

Step 3. Select **Assay #02_Trypan Blue Viability** from **Assay SETUP** drop-down menu:

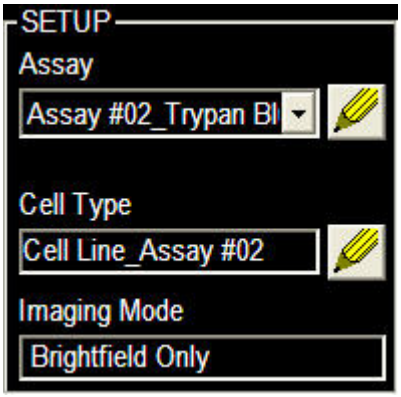
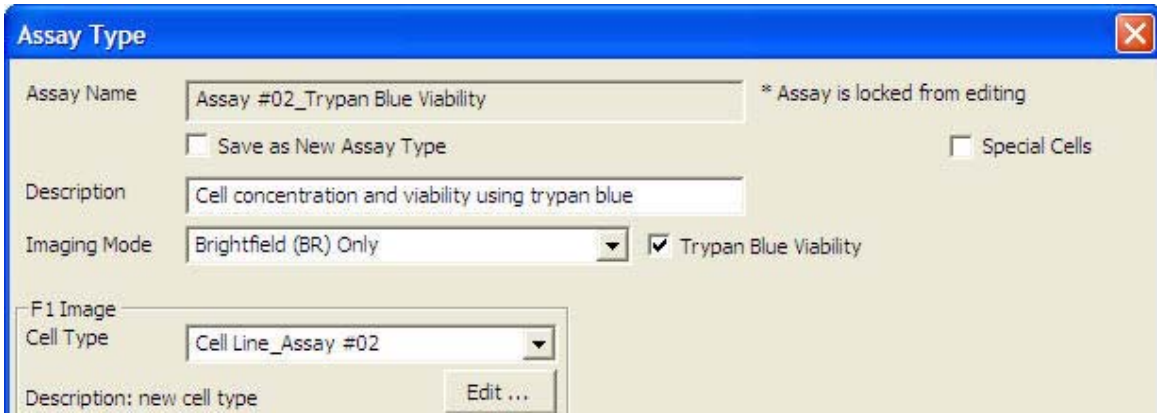


Image mode: Brightfield Only

To review **Assay Type** details: click **Edit Assay Button** (The yellow pencil next to **Assay SETUP**)



Check **Trypan Blue Viability**

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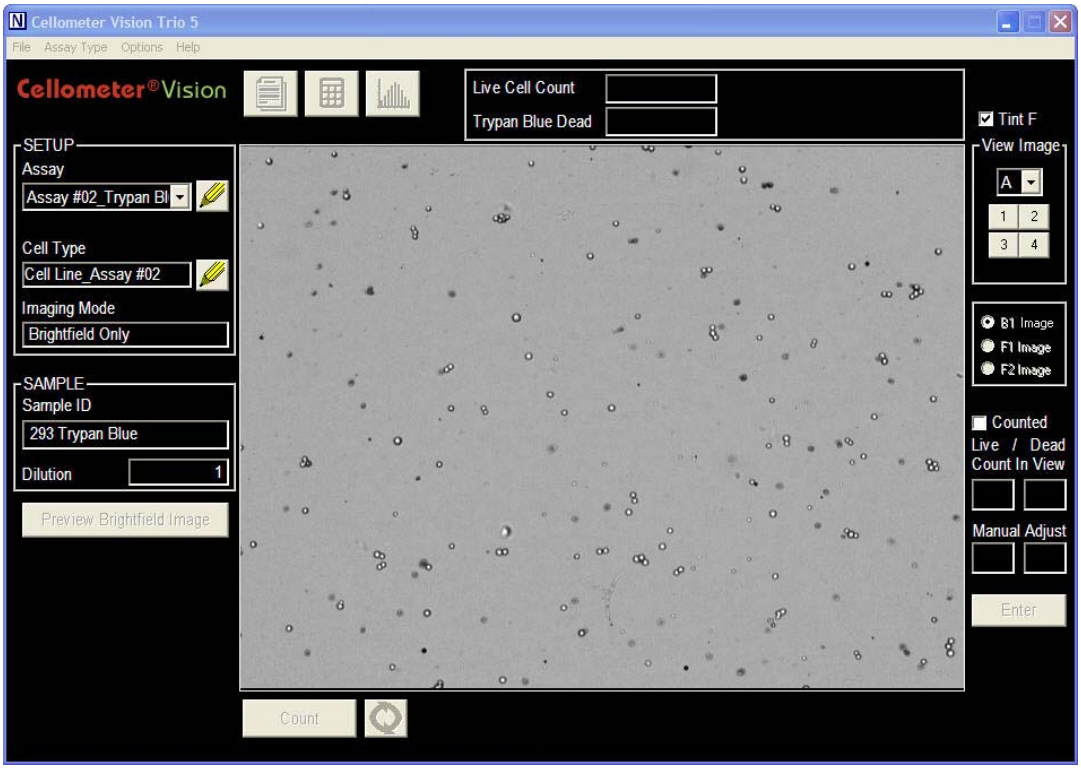
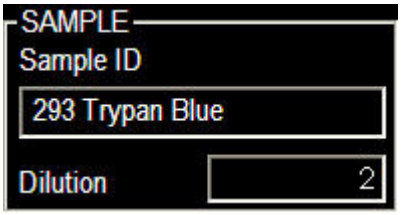
Step 4. Click **'Preview Brightfield Image'**:



Step 5. Input **Dilution factor** (Dilution = 2 if one part sample is added to one part trypan blue.) This will ensure that counting results reflect cell concentration of original sample.



Step 6. Adjust focus if necessary

Step 7. Click **'Count'**

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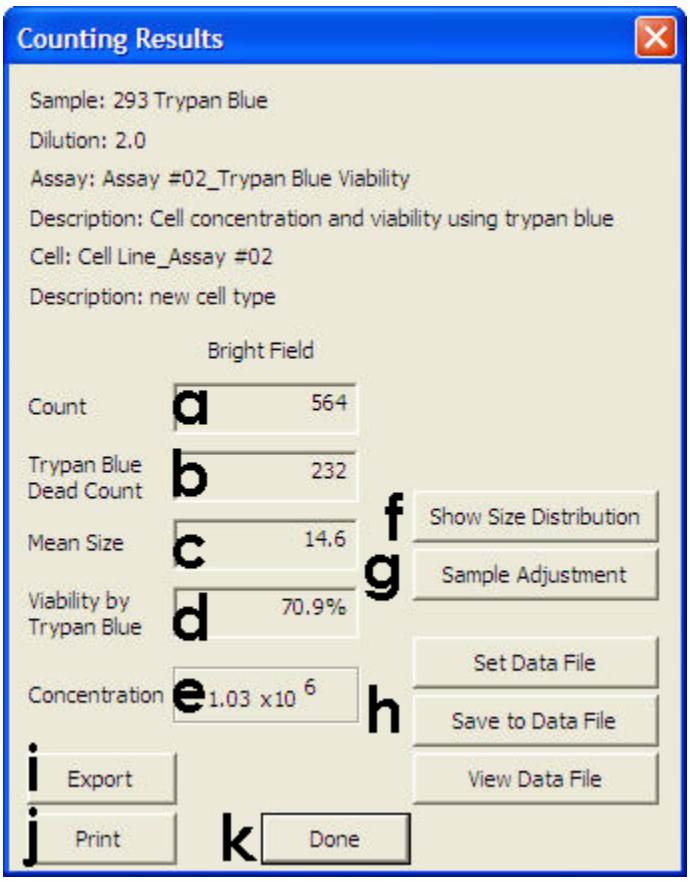
Step 8. Review **Counting Results** and display data as needed:



a. Count: *total number of cells counted*
b. Trypan Blue Dead Count: *number of trypan blue positive (dead) cells*
c. Mean Size: *mean diameter of the live cells*
d. Viability by Trypan Blue: *percentage of live cells out of total number of cells measured using trypan blue method*
e. Concentration: *live cell concentration (/mL)*
f. Show Size Distribution: *display cell size histogram*
g. Sample Adjustment: *launch sample adjustment calculator*
h. Save to Data File: *record counting results in a table*
i. Export: *stores all raw data, assay parameters and graphs in an Excel file*
j. Print: *send the current counting results to a printer*
k. Done: *closes counting results window*

Note: After clicking **'Done'** *the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button:*

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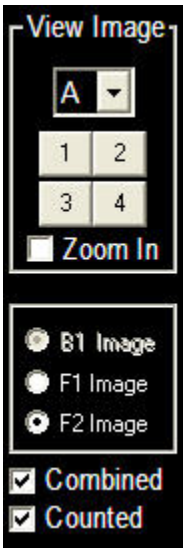
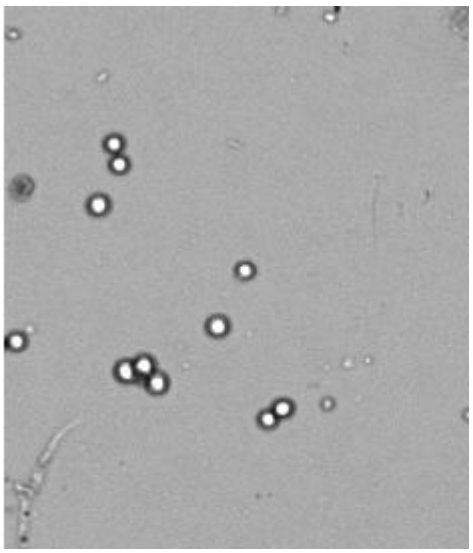
Step 9. Review Images on-screen

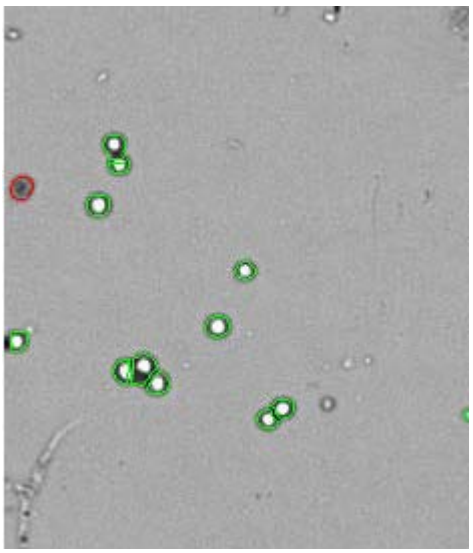


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Cell image before counting



B. Cell image after counting. Green outline indicates live cells and red outline indicates trypan blue positive dead cells.

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Assay #03 GFP Transfection Rate

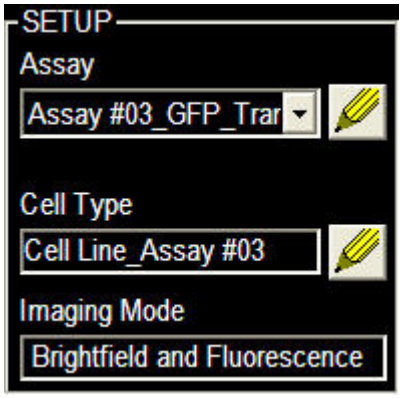
Transfection efficiency is often determined by using a GFP marker (either on the vector of interest or co-transfected with the vector). After taking brightfield and fluorescent images of the transfected sample, all cells are counted in brightfield and fluorescent images. Transfection efficiency is computed by dividing the number of fluorescent cells by the total number of cells.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Trypsinize attached cells

Step 2. Load 20µL in one side of the cell counting chamber and insert into instrument

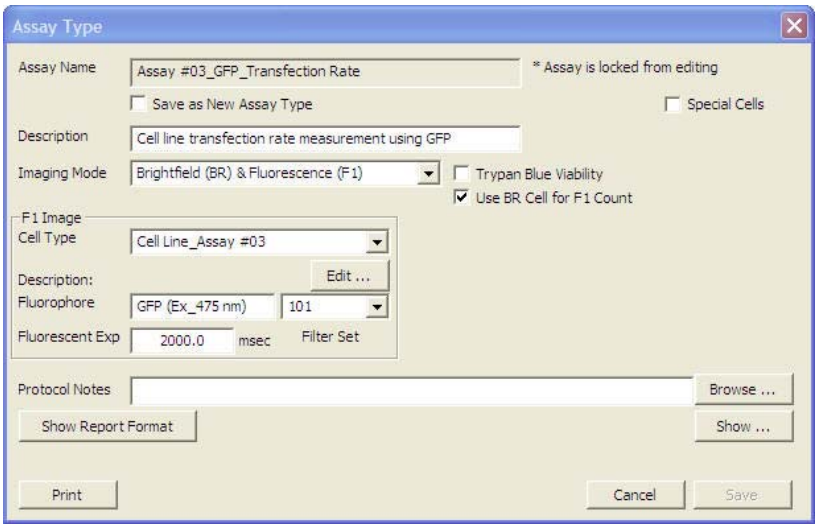
Step 3. Select **Assay #03_GFP_Transfection Rate** from **Assay SETUP** drop-down menu:



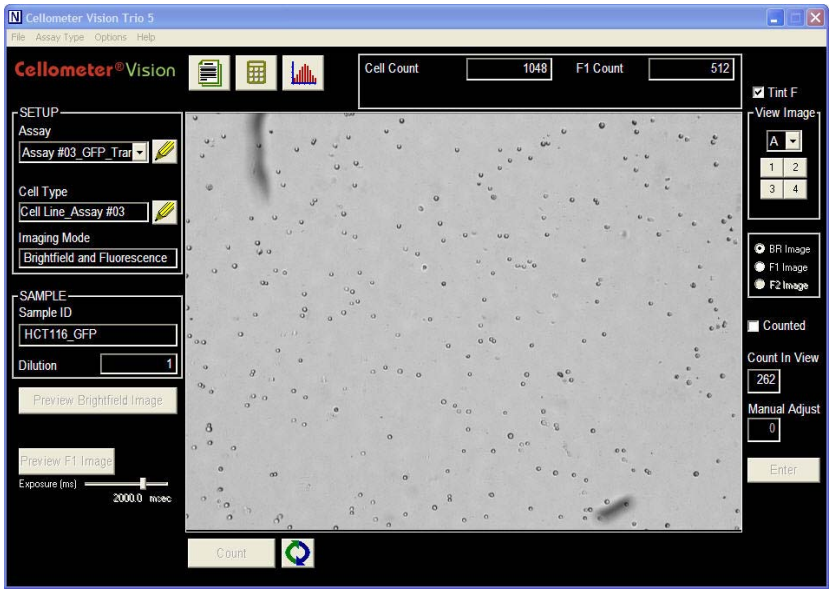
Cell Type: HCT is displayed.
Imaging Mode: Brightfield and Fluorescence

The brightfield imaging mode is used to measure total number of cells and cell size.
The fluorescence imaging mode is used to measure fluorescence positive cells.

Both Cell Type and Imaging Mode are defined in the Assay parameters, and can be edited from the main screen by clicking on the pencil icon.

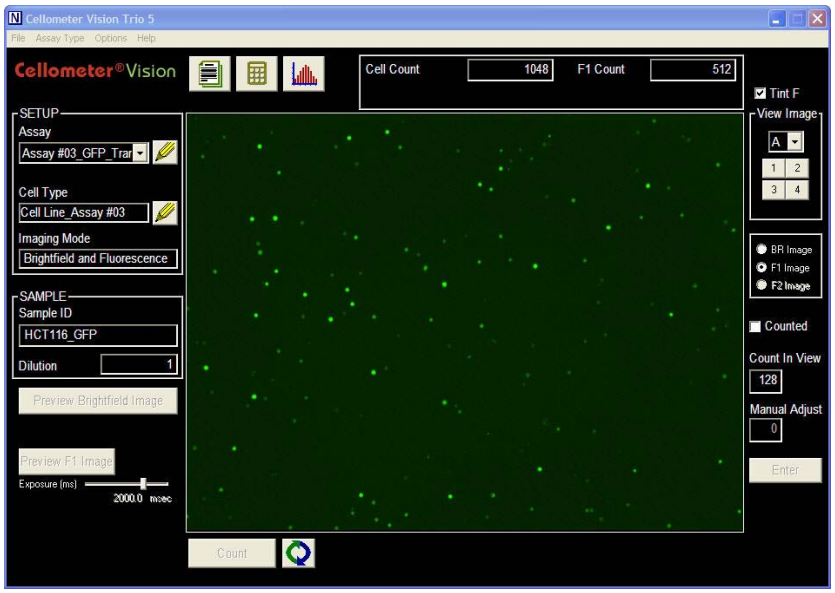


Step 4. Click **'Preview brightfield Image'**



Step 5. Adjust Focus if necessary

Step 6. Click **'Preview F1 Image'** (Fluorescence)



Step 7. Click **'Count'**

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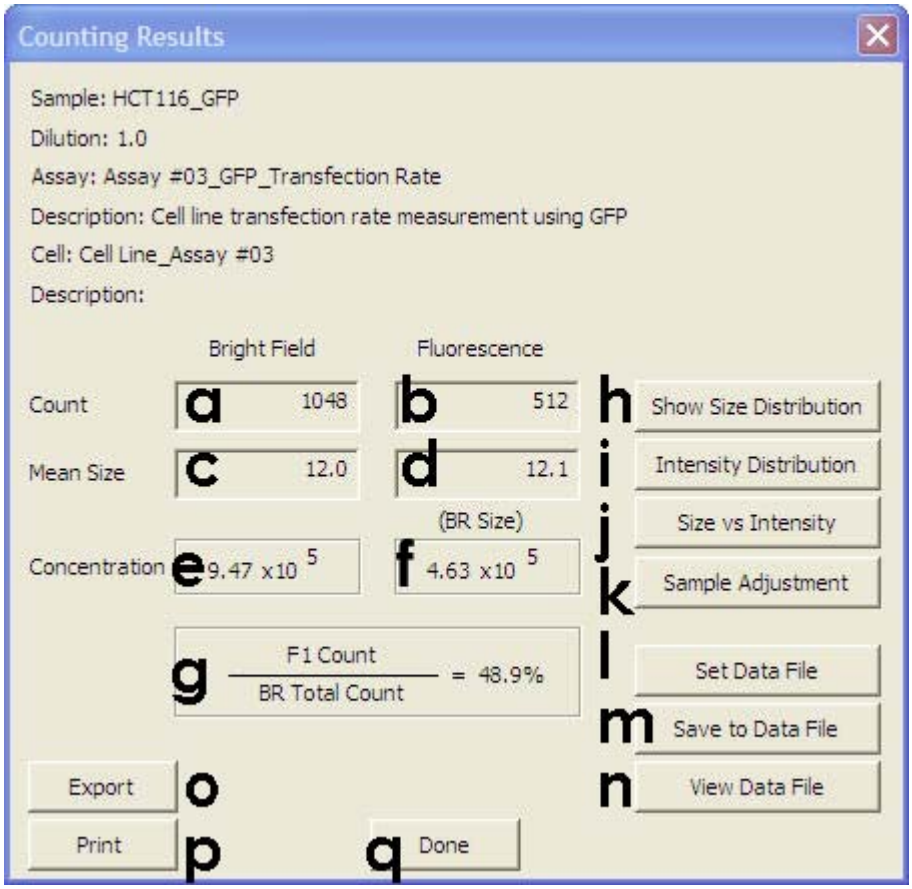
Step 8. Review **Counting Results** and display data as needed:



a. ***Count / brightfield:*** *total number of cells counted in the brightfield image*
b. ***Count / Fluorescence:*** *number of fluorescence positive cells counted*
c. ***Mean Size / brightfield:*** *mean diameter of cells measured in brightfield*
d. ***Mean Size / Fluorence:*** *mean diameter of fluorescence positive cells*
e. ***Concentration / Bright Field:*** *total cell concentration (/mL)*
f. ***Concentration / Fluorescence:*** *fluorescence cell concentration (/mL)*
g. ***F1 Count / BR Total Count:*** *transfection rate*

h. Show Size Distribution: *displays cell size histogram generated using brightfield image*
i. Intensity Distribution: *displays fluorescence cell intensity histogram*
j. Size vs Intensity: *displays fluorescence intensity vs. cell size scatterplot*
k. Sample Adjustment: *calculates adjustment for target concentration or target number of cells*
l. Set Data File: *creates a data file to store cell counting table*
m. Save to Data File: *records counting results in the cell counting table*
n. View Data File: *displays currently selected cell counting table*
o. Export: *stores all raw data, assay parameters and graphs in an Excel file*
p. Print: *sends the current counting results to a printer*
q. Done: *closes counting results window*
*Note: After clicking '**Done**' the Counting Results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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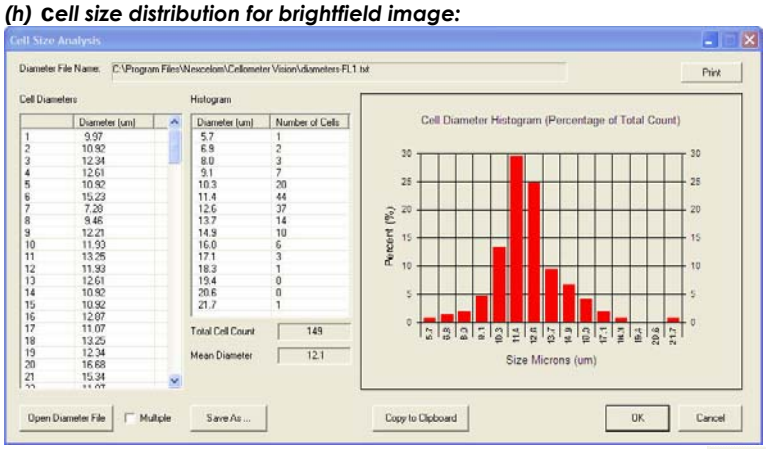
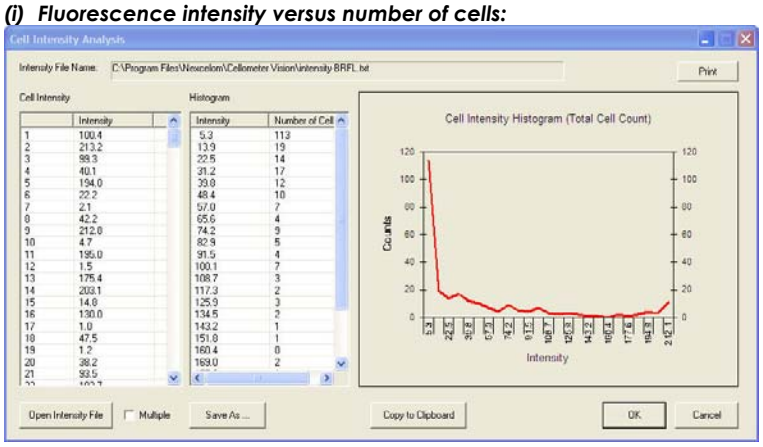
(h) Cell size distribution for brightfield image:

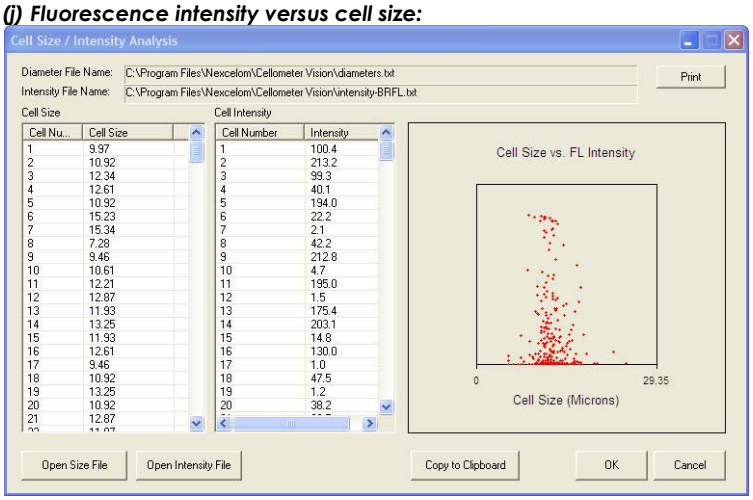


NOTE: *this can be instantly displayed at any time by clicking on the* *icon.*

(i) Fluorescence intensity versus number of cells:



(j) Fluorescence intensity versus cell size:



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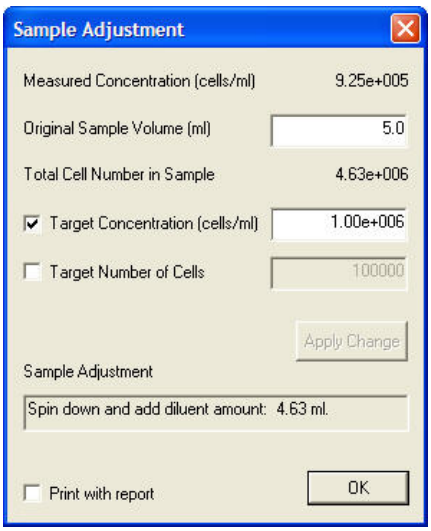
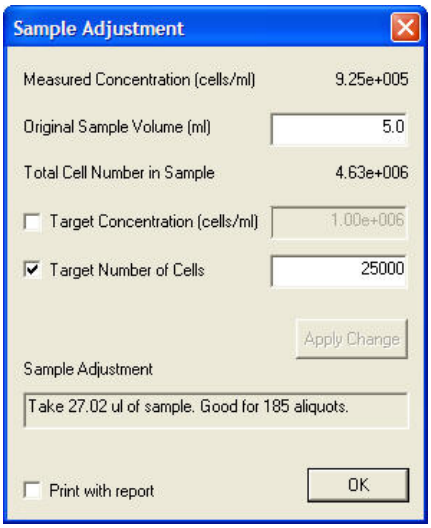
(k) Sample Adjustment Calculator:

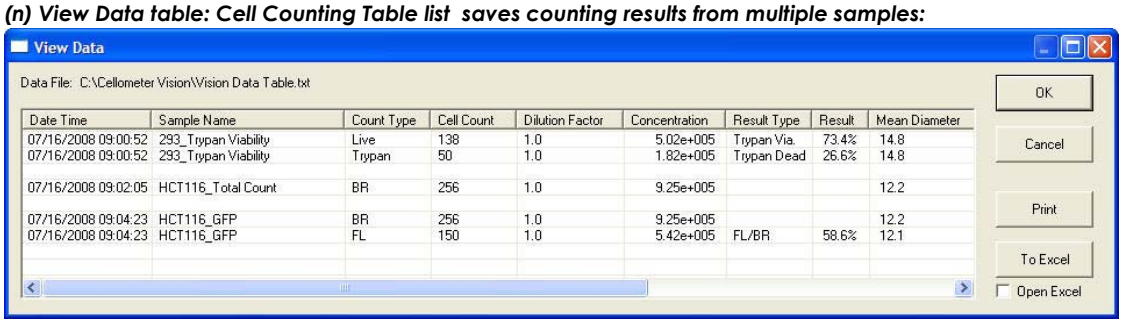


***Calculator #1**: input target cell concentration and calculate adjustment*



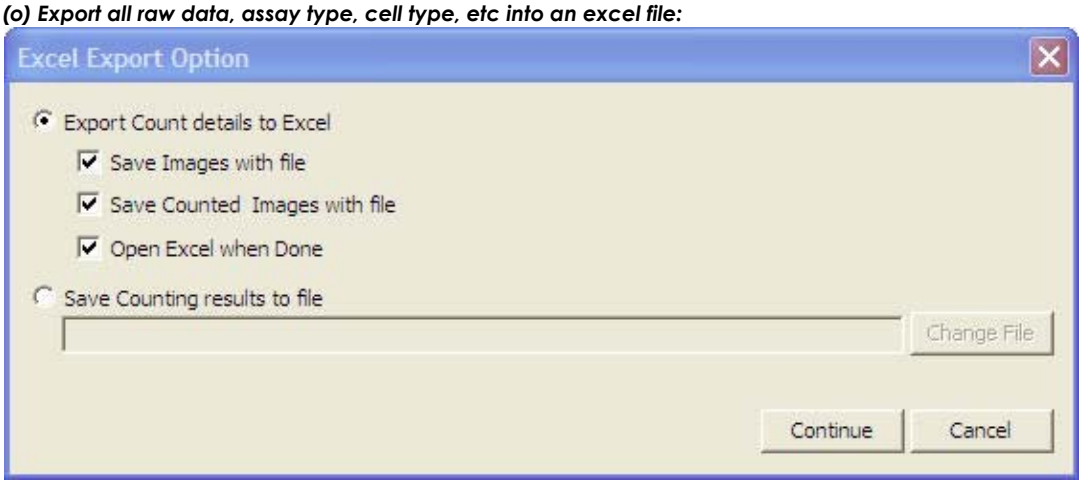
***Calculator #2:** input target number of needed. Amount of sample to add and the number of aliquots will be calculated*

(n) View Data table: Cell Counting Table list saves counting results from multiple samples:



Date Time	Sample Name	Count Type	Cell Count	Dilution Factor	Concentration	Result Type	Result	Mean Diameter
07/16/2008 09:00:52	293_Trypan Viability	Live	138	1.0	5.02e+005	Trypan Via.	73.4%	14.8
07/16/2008 09:00:52	293_Trypan Viability	Trypan	50	1.0	1.82e+005	Trypan Dead	26.6%	14.8
07/16/2008 09:02:05	HCT116_Total Count	BR	256	1.0	9.25e+005			12.2
07/16/2008 09:04:23	HCT116_GFP	BR	256	1.0	9.25e+005			12.2
07/16/2008 09:04:23	HCT116_GFP	FL	150	1.0	5.42e+005	FL/BR	58.6%	12.1

(o) Export all raw data, assay type, cell type, etc into an excel file:



NOTE: User can select which items get exported to Excel.

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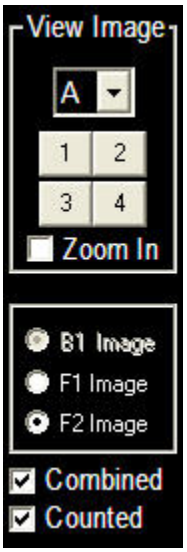
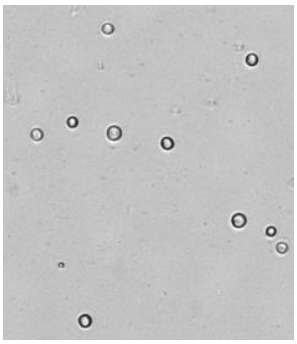
Step 9. Review Images on-screen

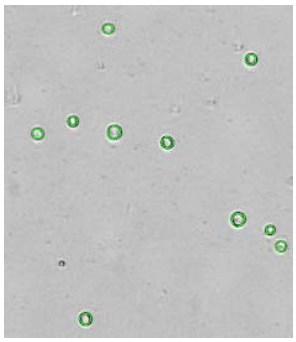


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. *Brightfield cell image*



B*. Counted cells indicated by the green outline*

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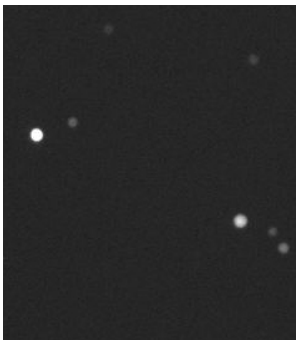

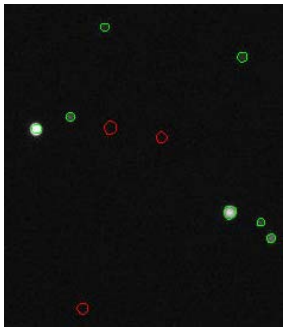

C. *Fluorescence cell image* ***D.*** *Counted fluorescence positive cells indicated by the green outline. The red outline indicated cells that are fluorescence negative.*

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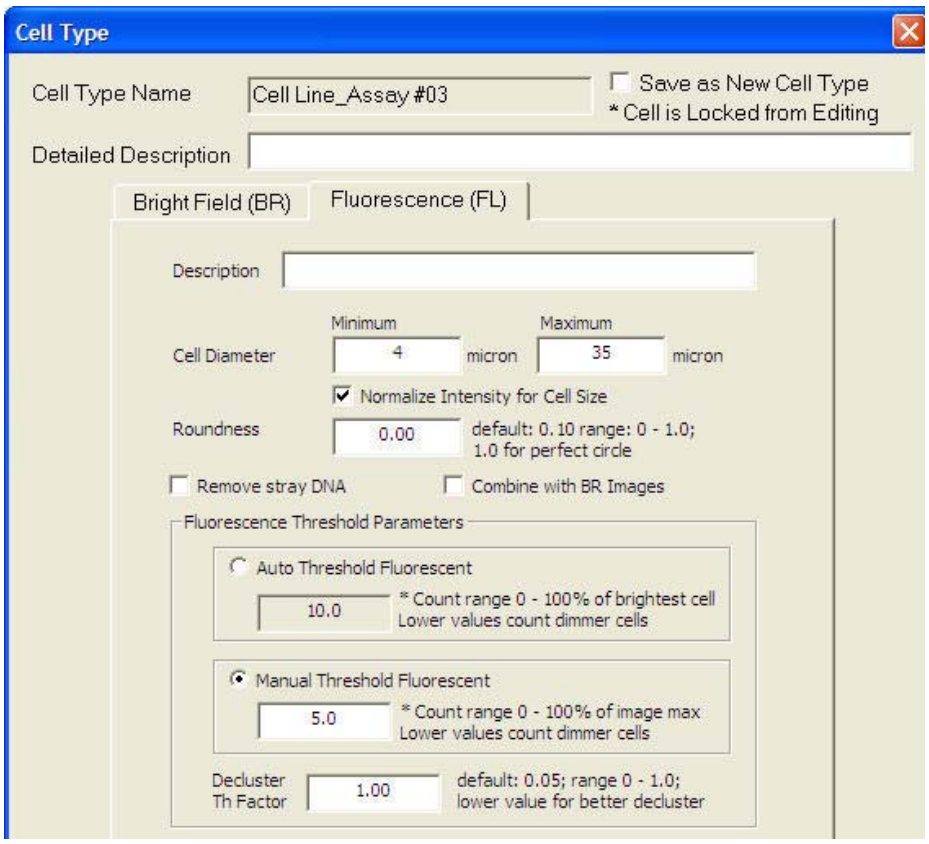
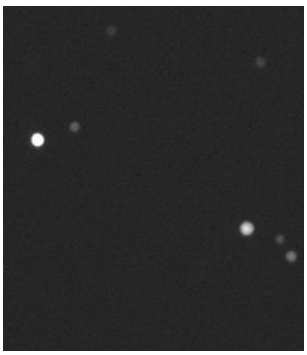
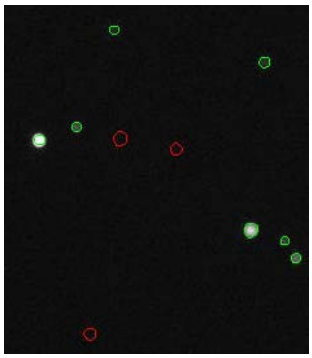
Step 10. To adjust fluorescence threshold (to determine which cells are counted as fluorescent positive or negative) adjust Cell Type Parameters by clicking on the pencil icon next to Cell Type.

Fluorescence Threshold is user defined to identify the brightness at which a cell is defined as positive:

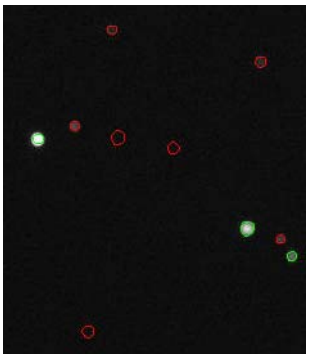




GFP fluorescence image



Threshold = 3%
F1 positive rate = 58.2%



Threshold = 20%
F1 positive rate = 28.9%
Increase threshold value counted only high intensity fluorescence as positive.

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Assay #04 RFP Transfection Rate

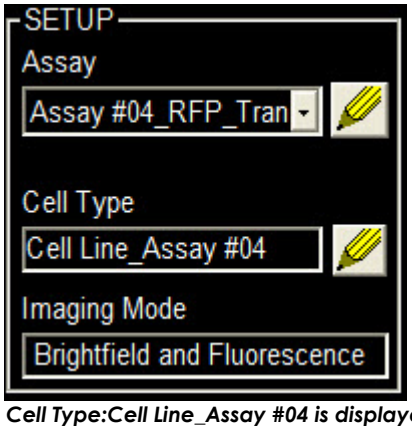
Transfection efficiency is often determined by using an RFP marker (either on the vector of interest or co-transfected with the vector). After taking brightfield and fluorescent images of the transfected sample, all cells are counted in brightfield and fluorescent images. Transfection efficiency is computed by dividing the number of fluorescent cells by the total number of cells.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Trypsinize attached cells

Step 2. Load 20µL in one side of the cell counting chamber and insert into instrument

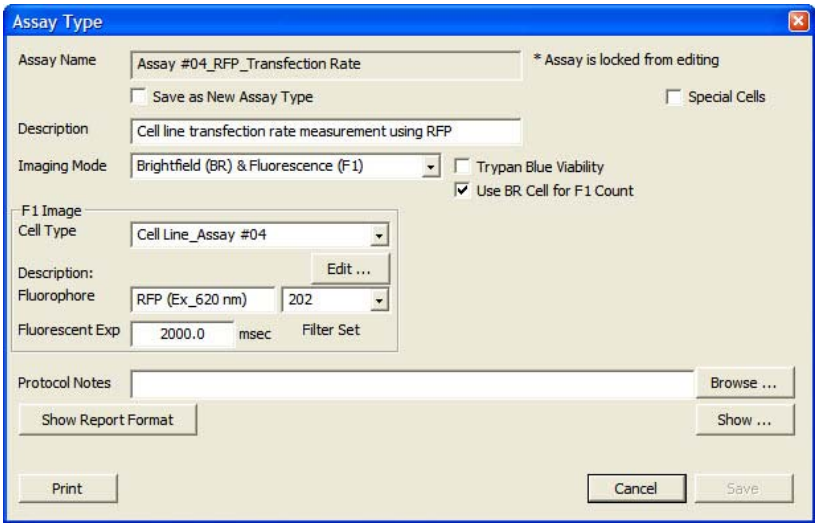
Step 3. Select **Assay #04 RFP Transfection Rate** from **Assay SETUP** drop-down menu:



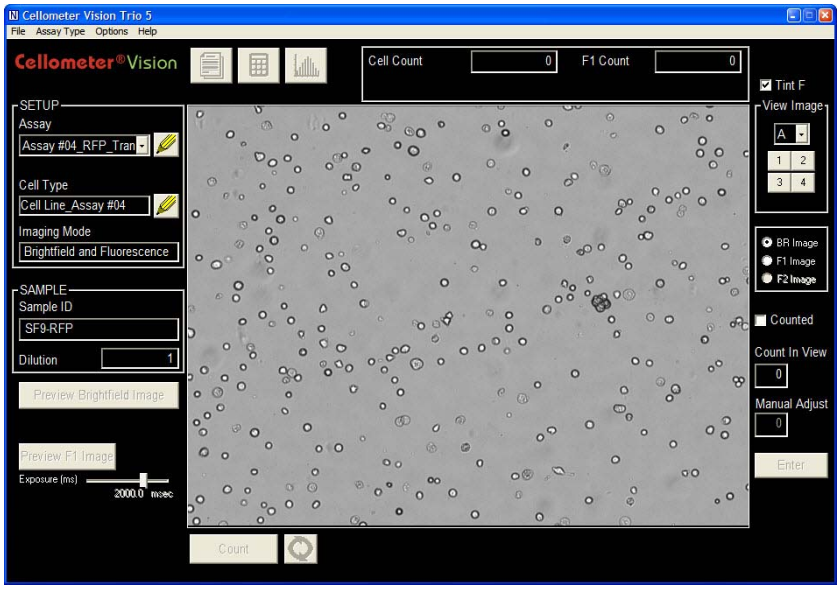
Cell Type:Cell Line_Assay #04 is displayed.
Imaging Mode: Brightfield and Fluorescence

The brightfield imaging mode is used to measure total number of cells and cell size.
The fluorescence imaging mode is used to measure fluorescence positive cells.

Both Cell Type and Imaging Mode are defined in the Assay parameters, and can be edited from the main screen by clicking on the pencil icon.

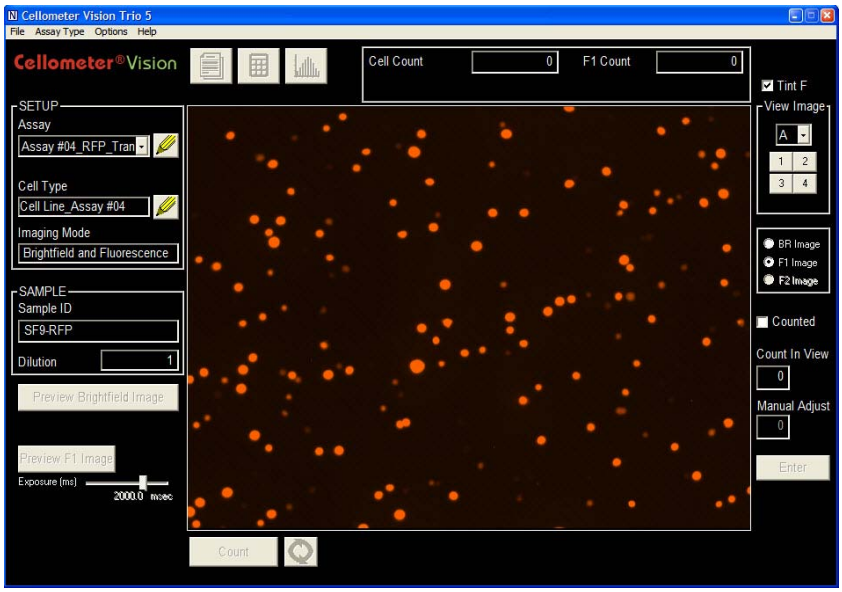


Step 4. Click **'Preview brightield Image'**



Step 5. Adjust Focus if necessary

Step 6. Click **'Preview F1 Image'** (Fluorescence)



Step 7. Click **'Count'**

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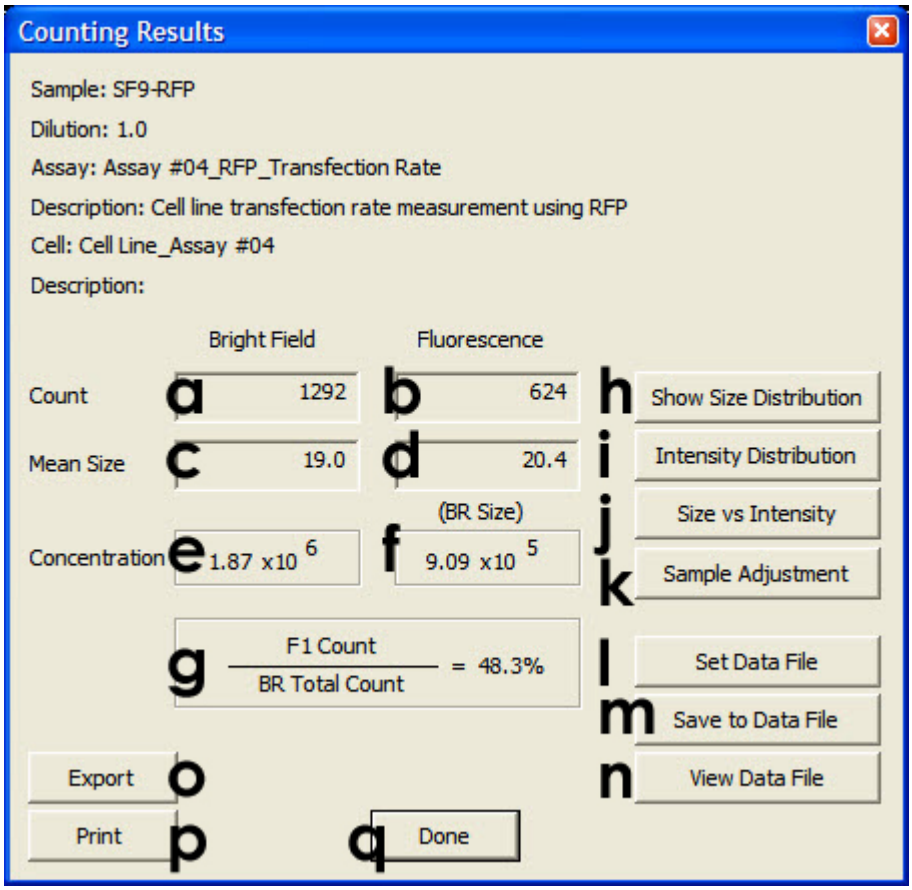
Step 8. Review **Counting Results** and display data as needed:



a. ***Count / brightfield:*** *total number of cells counted in the brightfield image*
b. ***Count / Fluorescence:*** *number of fluorescence positive cells counted*
c. ***Mean Size / brightfield:*** *mean diameter of cells measured in brightfield*
d. ***Mean Size / Fluorence:*** *mean diameter of fluorescence positive cells*
e. ***Concentration / Bright Field:*** *total cell concentration (/mL)*
f. ***Concentration / Fluorescence:*** *fluorescence cell concentration (/mL)*
g. ***F1 Count / BR Total Count:*** *transfection rate*

h. Show Size Distribution: *displays cell size histogram generated using brightfield image*
i. Intensity Distribution: *displays fluorescence cell intensity histogram*
j. Size vs Intensity: *displays fluorescence intensity vs. cell size scatterplot*
k. Sample Adjustment: *calculates adjustment for target concentration or target number of cells*
l. Set Data File: *creates a data file to store cell counting table*
m. Save to Data File: *records counting results in the cell counting table*
n. View Data File: *displays currently selected cell counting table*
o. Export: *stores all raw data, assay parameters and graphs in an Excel file*
p. Print: *sends the current counting results to a printer*
q. Done: *closes counting results window*
*Note: After clicking '**Done**' the Counting Results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button:*

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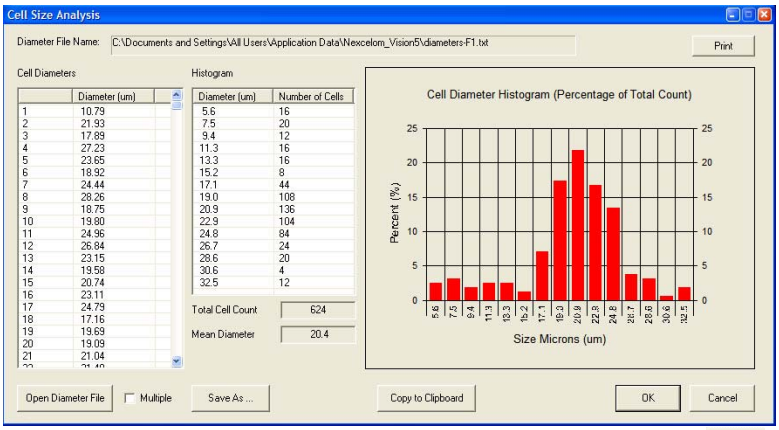
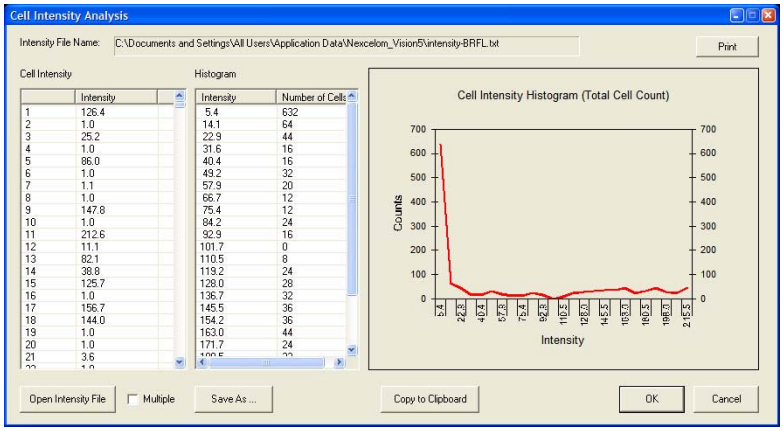
(h) Cell size distribution for brightfield image:

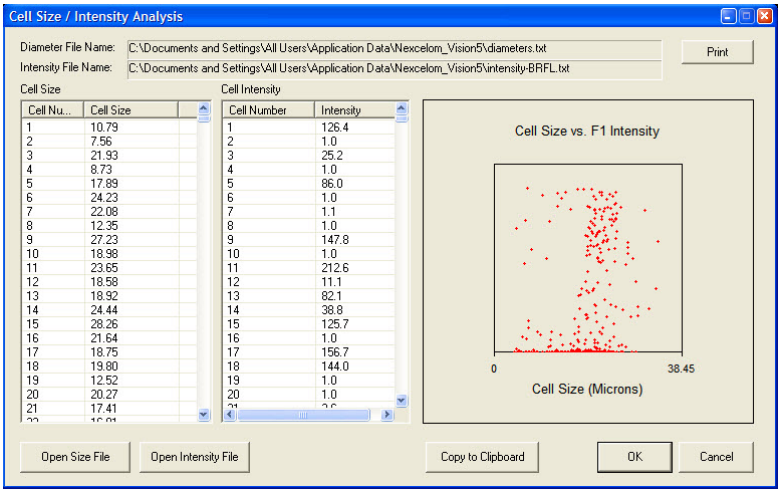


NOTE: *this can be instantly displayed at any time by clicking on the* *icon.*

(i) Fluorescence intensity versus number of cells:



(j) Fluorescence intensity versus cell size:



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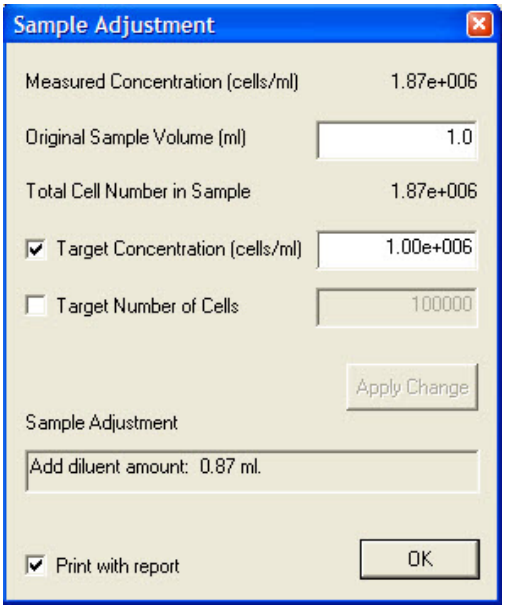
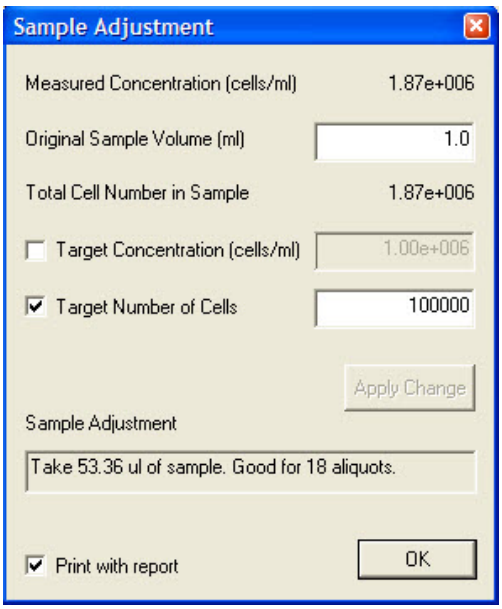
(*k) Sample Adjustment Calculator:*



Calculator #1: *input target cell concentration and calculate adjustment*



Calculator #2: *input target number of needed. Amount of sample to add and the number of aliquots will be calculated*

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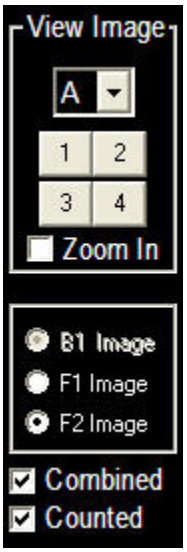
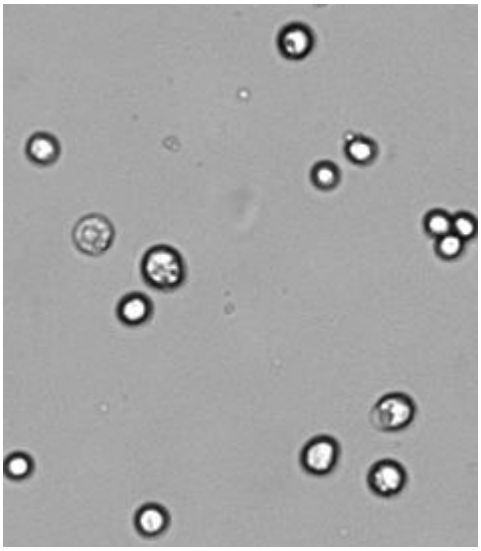
Step 9. Review Images on-screen

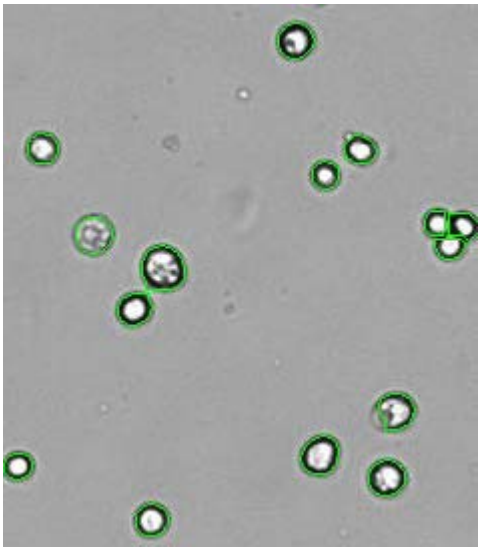


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Brightfield cell image



B. Counted cells

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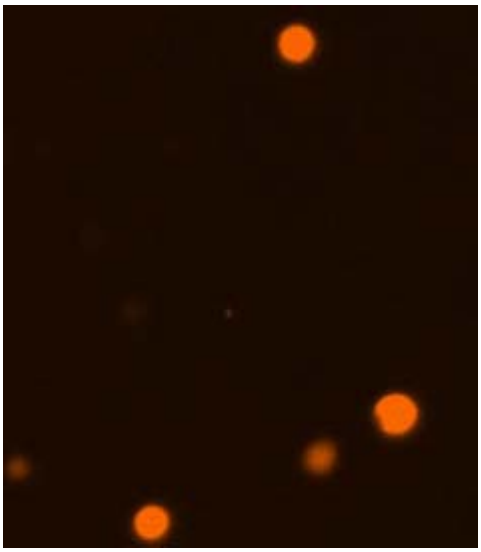
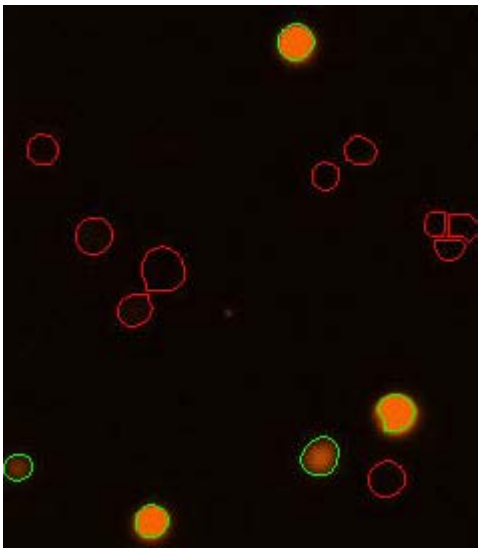


C. RFP positive cell image



D. Green outline indicates counted RFP positive cells

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Assay #05 YFP Transfection Rate

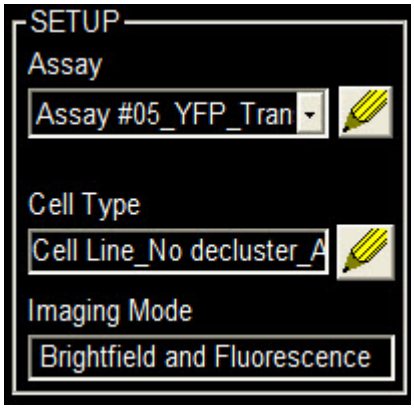
Transfection efficiency is often determined by using a YFP marker (either on the vector of interest or co-transfected with the vector). After taking brightfield and fluorescent images of the transfected sample, all cells are counted in brightfield and fluorescent images. Transfection efficiency is computed by dividing the number of fluorescent cells by the total number of cells.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Trypsinize attached cells

Step 2. Load 20µL in one side of the cell counting chamber and insert into instrument

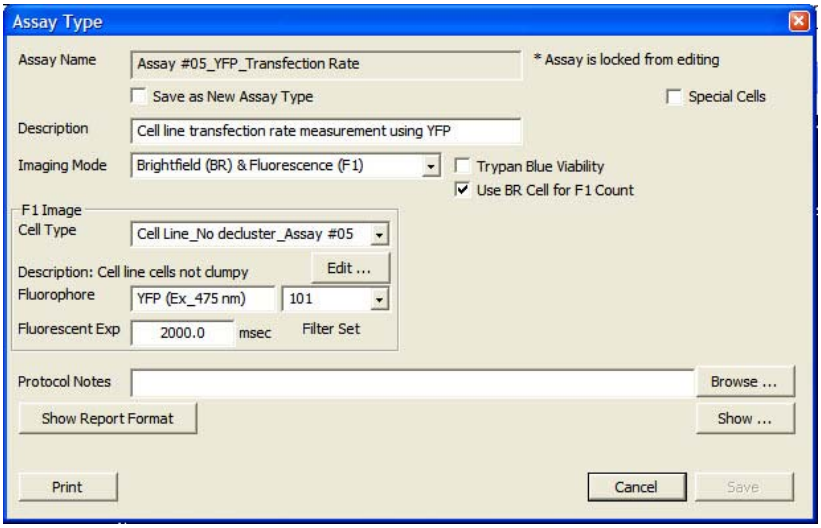
Step 3. Select **Assay #05 YFP Transfection Rate** from **Assay SETUP** drop-down menu:



Cell Type: Cell Line_No decluster_Assay #05 is displayed.
Imaging Mode: Brightfield and Fluorescence

The brightfield imaging mode is used to measure total number of cells and cell size.
The fluorescence imaging mode is used to measure fluorescence positive cells.

Both Cell Type and Imaging Mode are defined in the Assay parameters, and can be edited from the main screen by clicking on the pencil icon.



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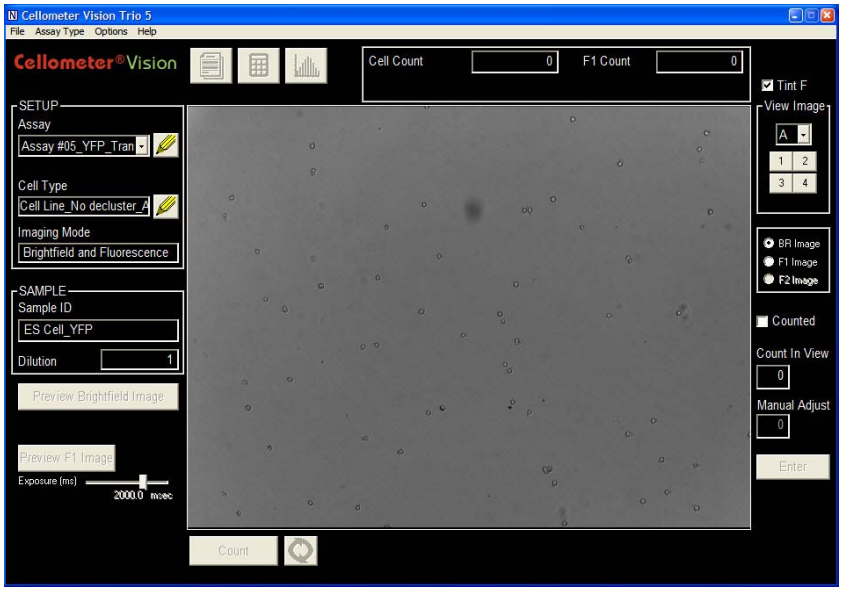
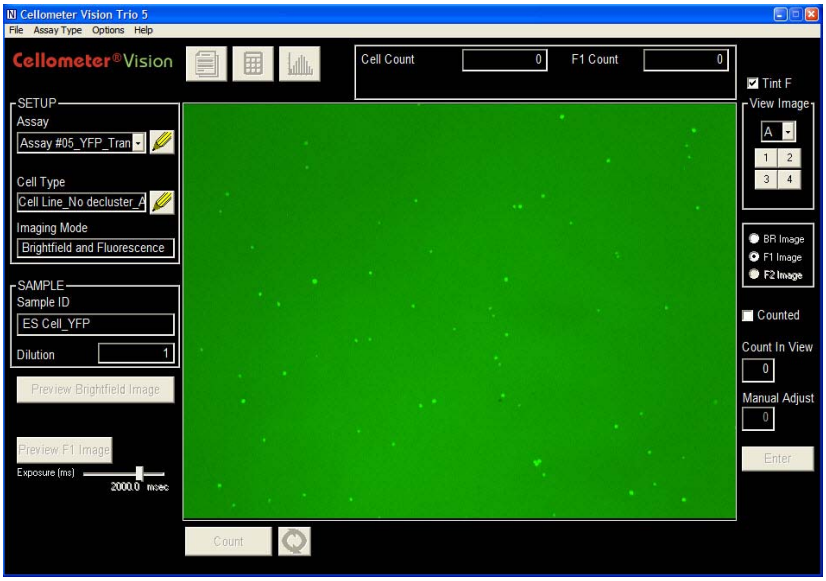
Step 4. Click **'Preview brightfield Image'**



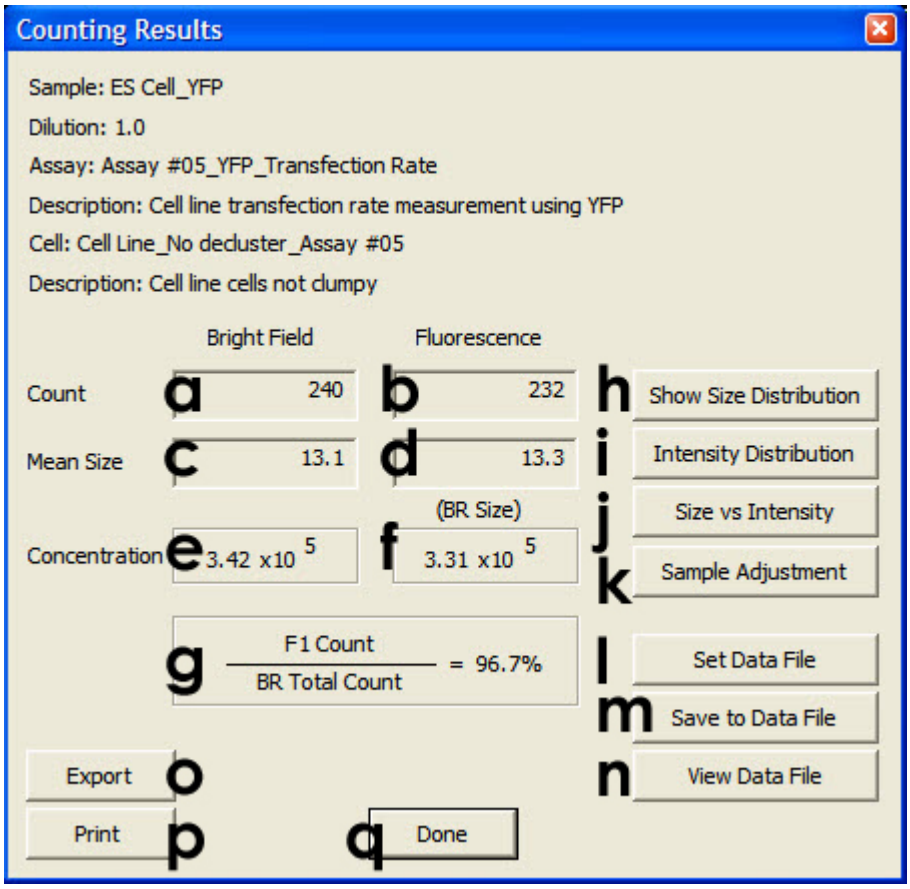
Step 5. Adjust Focus if necessary

Step 6. Click **'Preview F1 Image'** (Fluorescence)



Step 7. Click **'Count'**

Step 8. Review **Counting Results** and display data as needed:



a. Count / brightfield: *total number of cells counted in the brightfield image*
b. Count / Fluorescence: *number of fluorescence positive cells counted*
c. Mean Size / brightfield: *mean diameter of cells measured in brightfield*
d. Mean Size / Fluorence: *mean diameter of fluorescence positive cells*
e. Concentration / Bright Field: *total cell concentration (/mL)*
f. Concentration / Fluorescence: *fluorescence cell concentration (/mL)*
g. F1 Count / BR Total Count: *transfection rate*

h. Show Size Distribution: *displays cell size histogram generated using brightfield image*
i. Intensity Distribution: *displays fluorescence cell intensity histogram*
j. Size vs Intensity: *displays fluorescence intensity vs. cell size scatterplot*
k. Sample Adjustment: *calculates adjustment for target concentration or target number of cells*
l. Set Data File: *creates a data file to store cell counting table*
m. Save to Data File: *records counting results in the cell counting table*
n. View Data File: *displays currently selected cell counting table*
o. Export: *stores all raw data, assay parameters and graphs in an Excel file*
p. Print: *sends the current counting results to a printer*
q. Done: *closes counting results window*
Note: After clicking **'Done'** *the Counting Results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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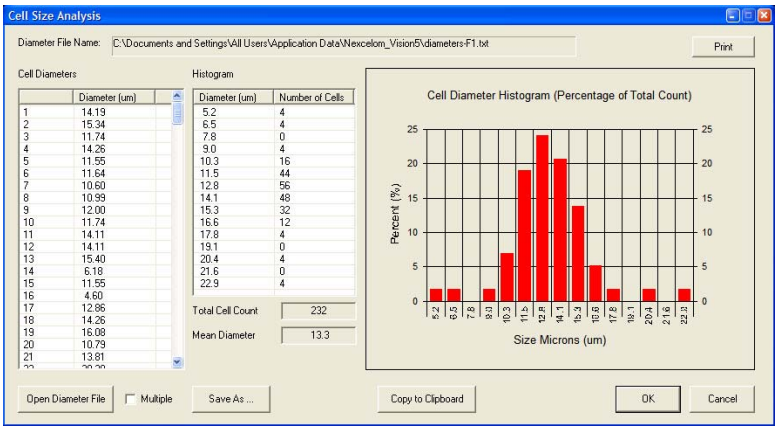
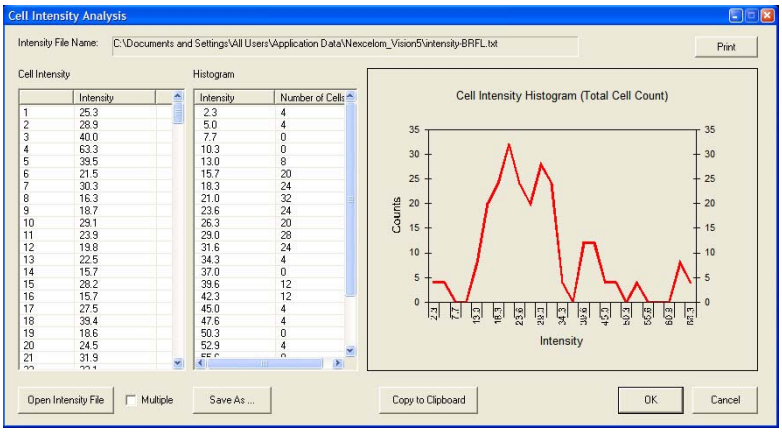
(h) Cell size distribution for brightfield image:

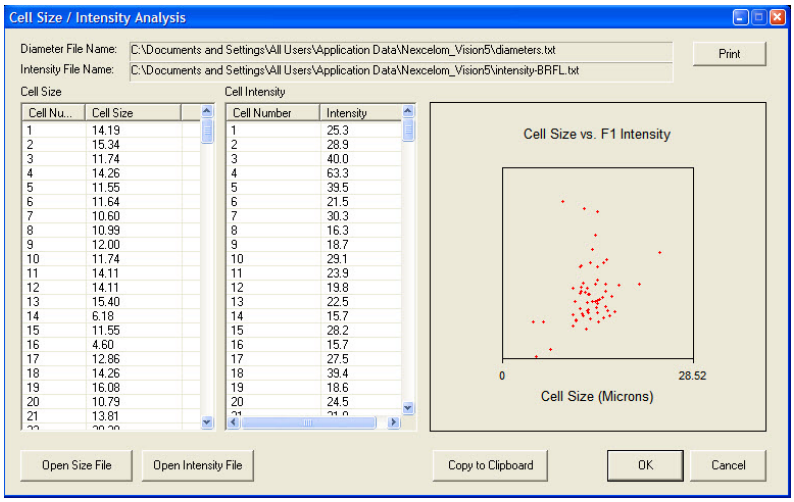


NOTE: *this can be instantly displayed at any time by clicking on the* *icon.*

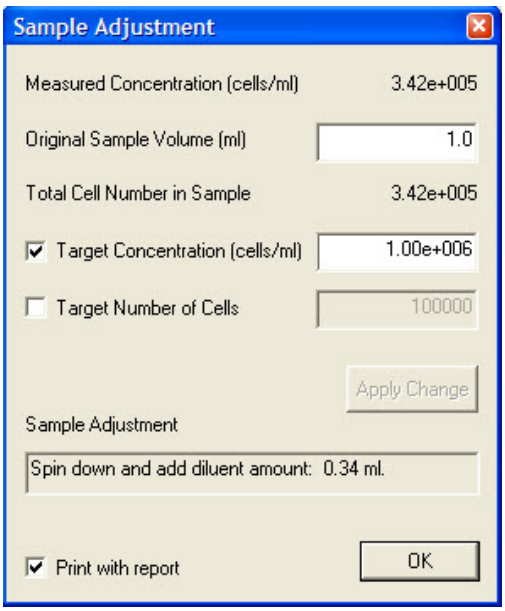
(i) Fluorescence intensity versus number of cells:



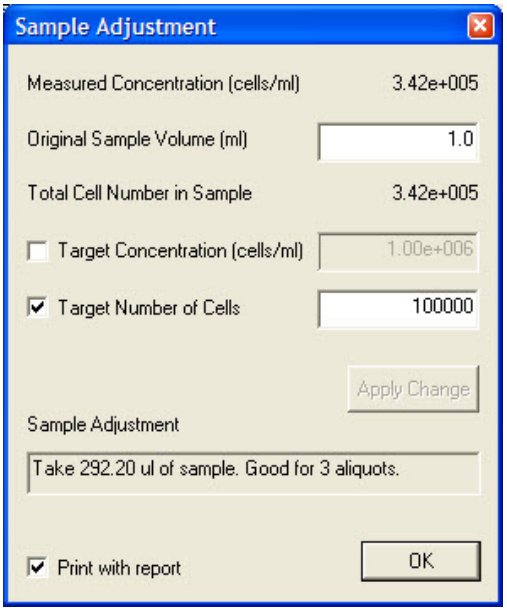
(j) Fluorescence intensity versus cell size:



(*k) Sample Adjustment Calculator:*



Calculator #1: *input target cell concentration and calculate adjustment*



Calculator #2: *input target number of needed. Amount of sample to add and the number of aliquots will be calculated*

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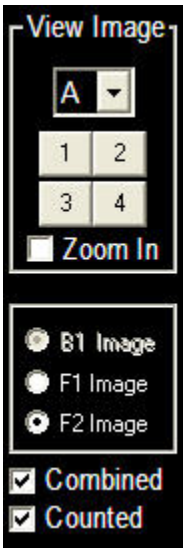
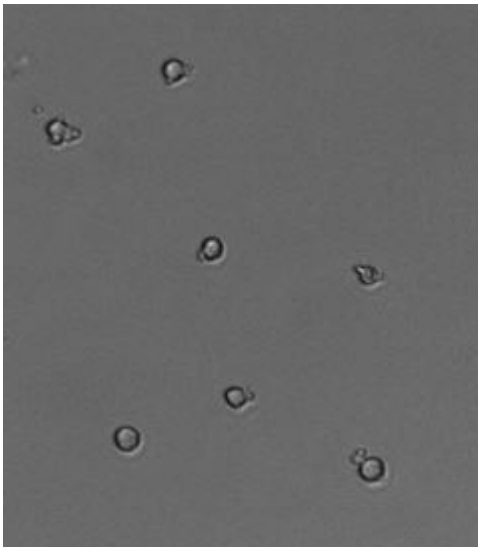
Step 9. Review Images on-screen

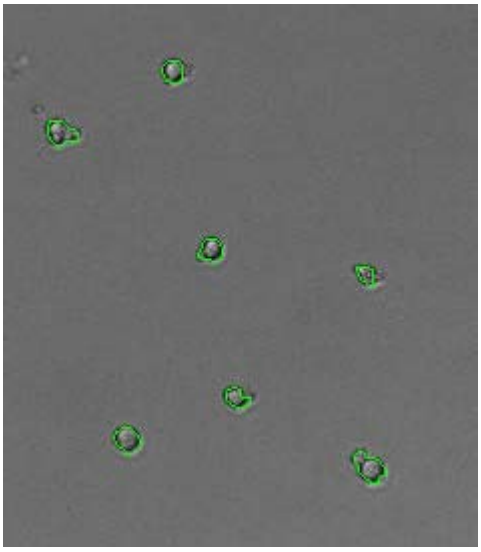


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Brightfield cell image



B. Counted cells

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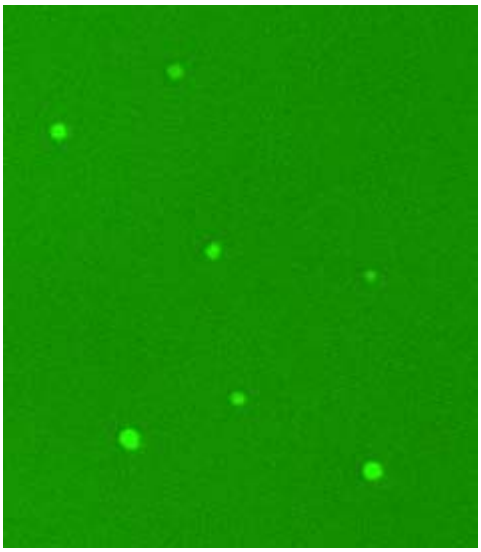
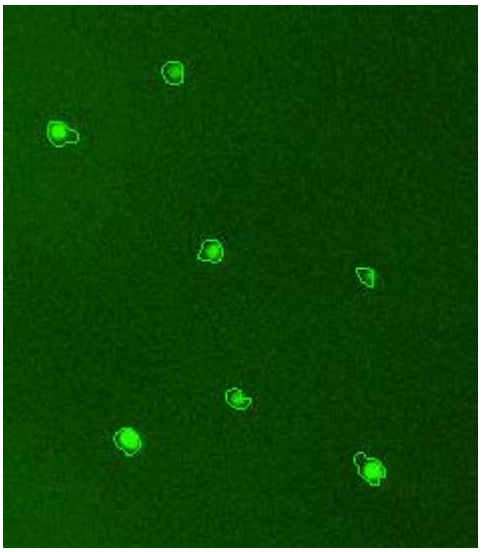


C. YFP positive cell image



D. Green outline indicates counted YFP positive cells

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Assay #06 White Blood Cell Concentration in Whole Blood with Acridine Orange Staining

Determining white blood cell counts in whole blood normally involves a lysis procedure to eliminate red blood cells. However, WBCs can be counted in whole blood without lysis of RBCs by staining the blood sample with Acridine orange, a nuclear stain that emits in the 'green' range. After taking 'green' fluorescent images of AO stained whole blood, all fluorescent cells are counted and their concentration in whole blood is determined. Brightfield images of the sample can be taken but are not used for WBC counting. (If RBC counting results are desired, the original blood sample can be diluted approximately 1:1000 and counted using only brightfield images, as in Assay #01)

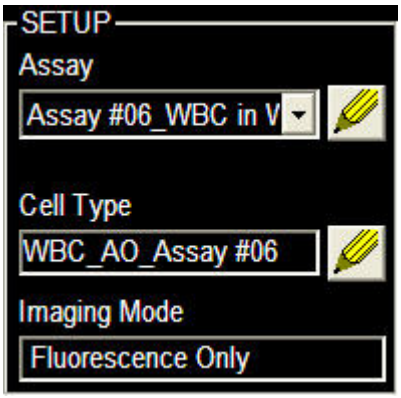
Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Dilute blood in PBS 1:10

Step 2. Mix cell samples with AO and incubate using the same method as when manually counting (blood concentration 1:20, AO concentration 2-10 mg/mL)

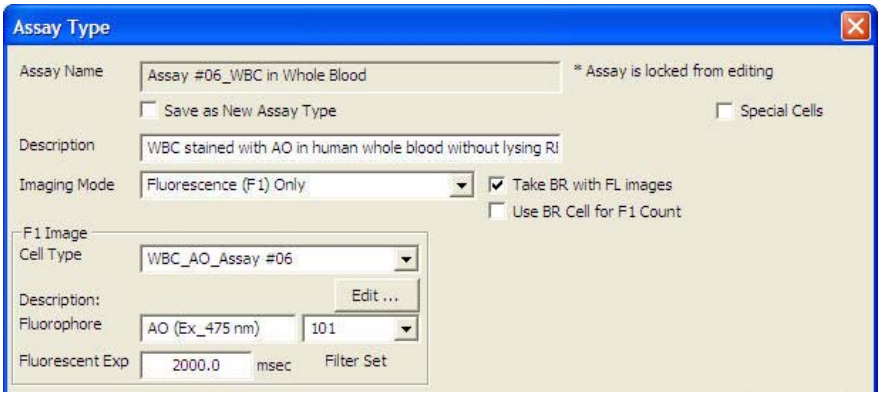
Step 3. Load 20µL AO stained cell sample in one side of the cell counting chamber and insert into instrument

Step 4. Select **Assay #06_WBC in Whole Blood** from the **Assay SETUP** drop-down menu:



Imaging mode: Fluorescence Only

Click on the yellow pencil on the right side of Assay selection to view Assay Type



Take BR with FL Image: check to take brightfield image of the same cell sample

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Use BR cell for F1 Count: uncheck to NOT use brightfield counting method

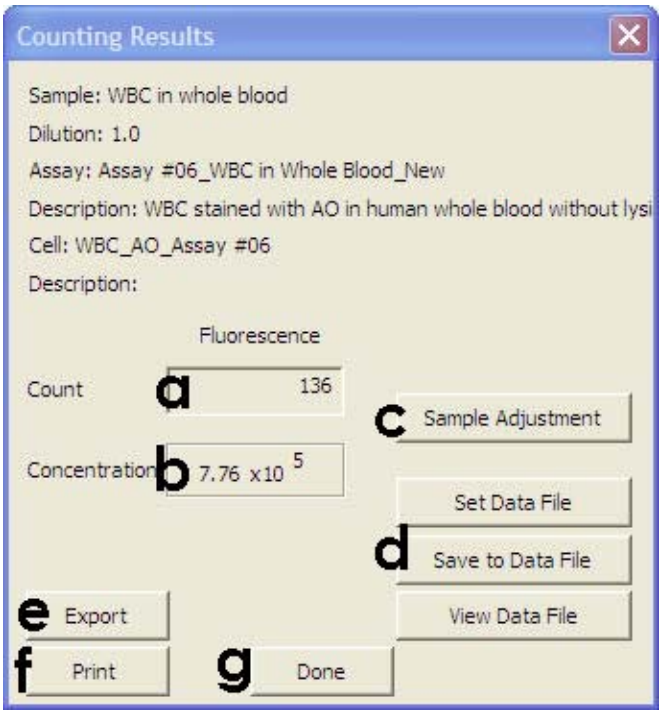
Step 5. Click **'Preview Brightfield Image'**

Step 6. Adjust focus if necessary

Step 7. Click **'Preview F1 Image'**

Step 8. Click **'Count'**

Step 9. Review **Counting Results** and display data as needed:



a. ***Count:*** *total number of white blood cells*
b. ***Concentration:*** *concentration of white blood cells*
c. ***Sample Adjustment:*** *calculates adjustment for target concentration or target number of cells*
d. ***Save to Data File:*** *record counting results in a table*
e. ***Export:*** *stores all raw data, assay parameters and graphs in an Excel file*
f. ***Print:*** *send current counting results to printer*
g. ***Done:*** *closes counting results window*
*Note: After clicking '**Done**' the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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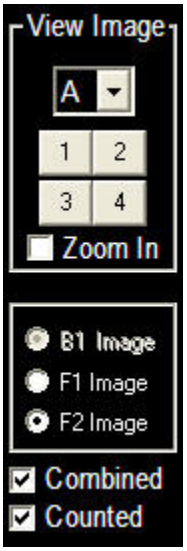
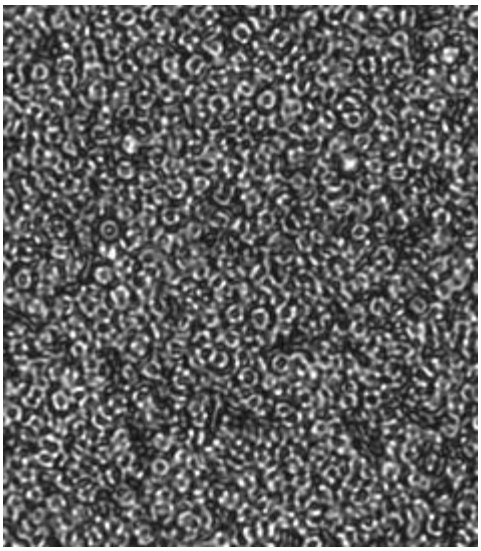
Step 10. Review Images on-screen



A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Brightfield image shows sample with crowded red blood cells.

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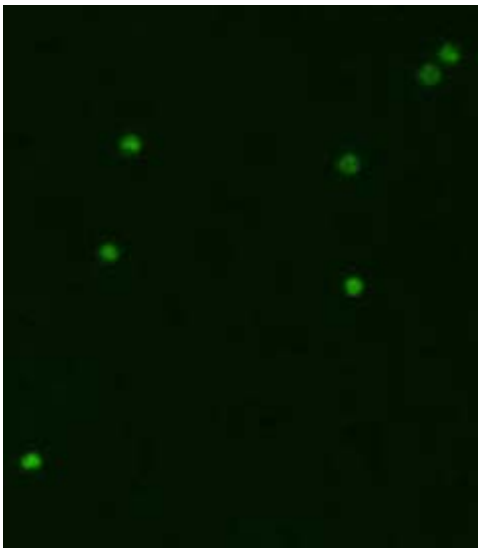
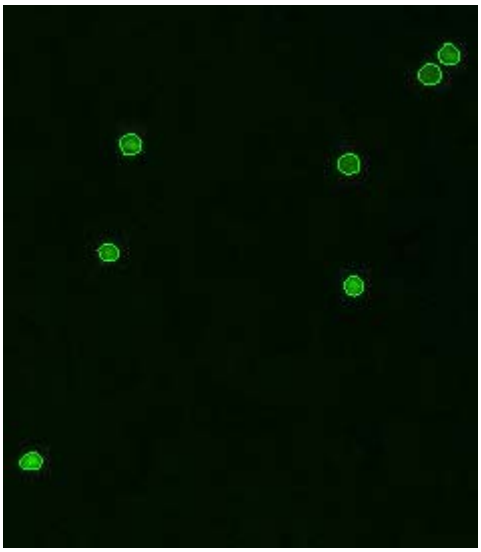


B. Fluorescent cell image before counting outline



C. Fluorescent cell image after counting. Green indicates counted white blood cells

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Assay #07 PBMC Concentration Using Acridine Orange Staining

PBMC preparations generally contain some RBCs making it difficult to accurately count only the PBMCs. RBCs can be eliminated to improve counting by using a lysis procedure. However, PBMCs can be selectively counted without lysis of RBCs by staining the PBMC preparation with Acridine orange, a nuclear stain that emits in the 'green' range. After taking 'green' fluorescent images, all fluorescent cells are counted and their concentration in the PBMC preparation is determined. Brightfield images of the sample can be taken but are not used for PBMC counting.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Mix cell sample with AO and incubate using the same method as when manually counting (AO concentration 2-10 mg/mL)

Step 2. Load 20µL in one side of the cell counting chamber and insert into instrument

Step 3. Select **Assay #07_PBMC with AO** from the **Assay SETUP** drop-down menu:

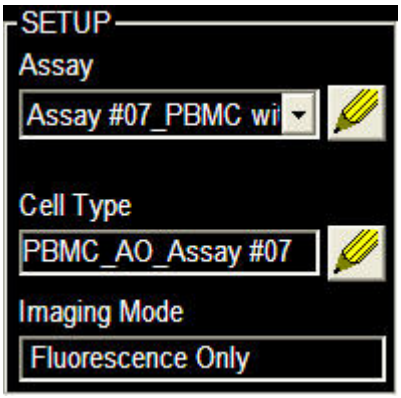
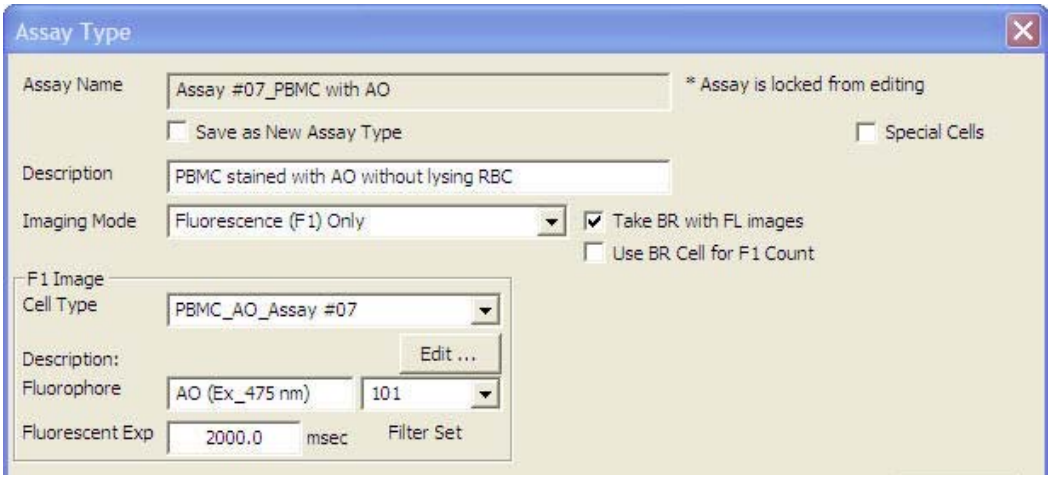


Image mode: Fluorescence Only

Click on the yellow pencil on the right side of Assay selection to view Assay Type



Take BR with FL Image: check to take brightfield image of the same cell sample
Use BR cell for F1 Count: uncheck to NOT use brightfield counting method

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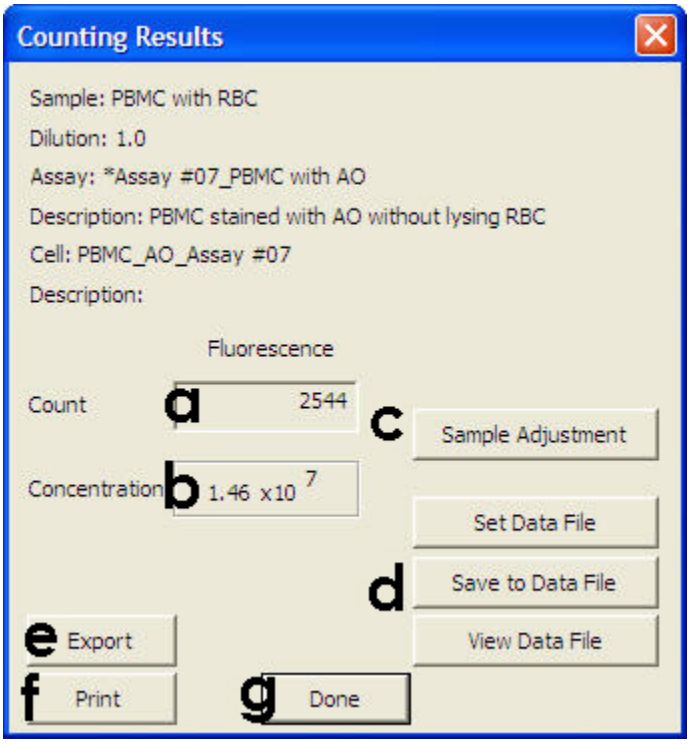
Step 4. Click **'Preview Brightfield Image'**

Step 5. Adjust focus if necessary

Step 6. Click **'Preview F1 Image'**

Step 7. Click **'Count'**

Step 8. Review **Counting Results** and display data as needed:



a. *Count:* *number PBMC counted*
b. *Concentration:* *PBMC concentration /mL*
c. ***Sample Adjustment:*** *calculates adjustment for target concentration or target number of cells*
d. ***Save to Data File:*** *record counting results in a table*
e. ***Export:*** *stores all raw data, assay parameters and graphs in an Excel file*
f. ***Print:*** *send current counting results to printer*
g. ***Done:*** *closes counting results window*
Note: After clicking **'Done'** *the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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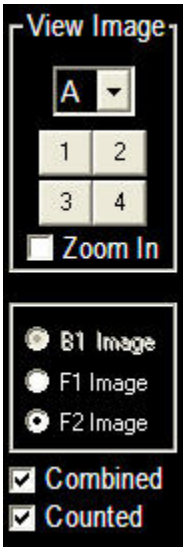
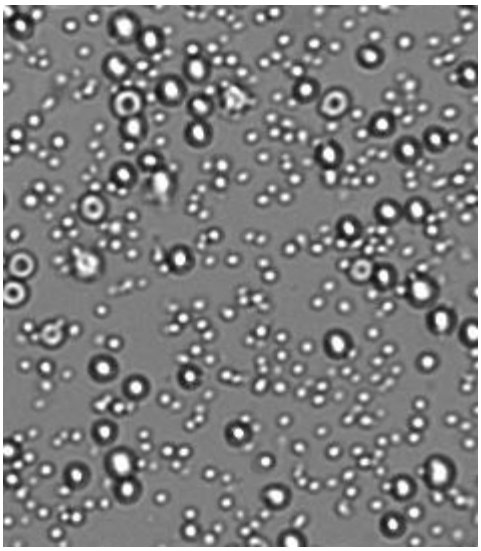
Step 9. Review Images on-screen



A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Brightfield image of PBMCs with contaminating red blood cells

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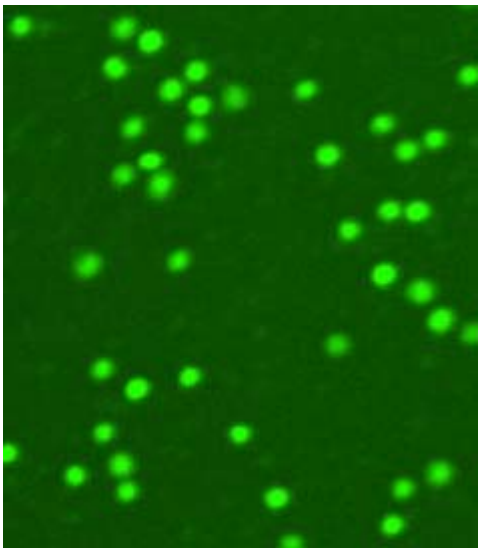
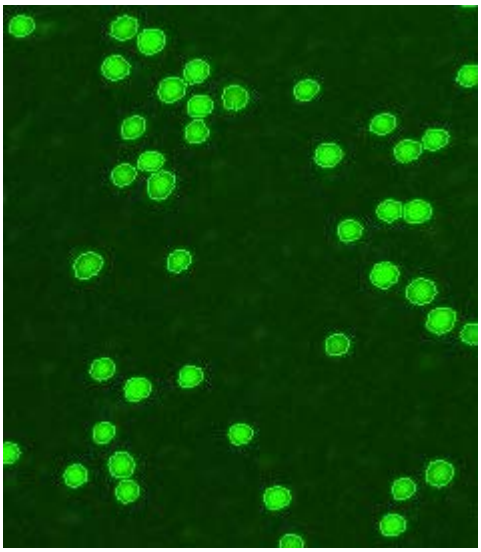


B. Fluorescence image of AO stained PBMCs



C. PBMCs after counting. Green outline indicates counted cells

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Assay #08 Cell Viability Using Propidium Iodide Staining

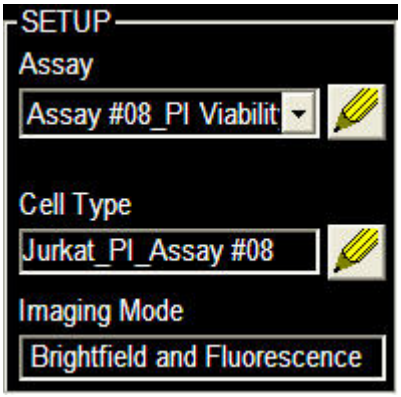
Propidium iodide is routinely used to determine cell viability. PI is a fluorescent stain that only penetrates dead cells and emits in the 'red' range (live cells are unaffected by PI). After taking both brightfield and ' red' fluorescent images of a PI stained sample, all cells (from brightfield channel) and dead cells (from 'red ' fluorescent channel) are counted to determine total and dead cell concentrations and compute the percent viability.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Mix cell samples with PI and incubate using the same method as when manually counting.

Step 2. Load 20µL stained cell sample in one side of the cell counting chamber and insert into instrument

Step 3. Select **Assay #08_PI Viability Jurkat** from the **Assay SETUP** drop-down menu:



Imaging mode: Brightfield and Fluorescence

Click on the yellow pencil on the right side of Assay selection to view Assay Type
Click "Show Report Format" to display all available data display options

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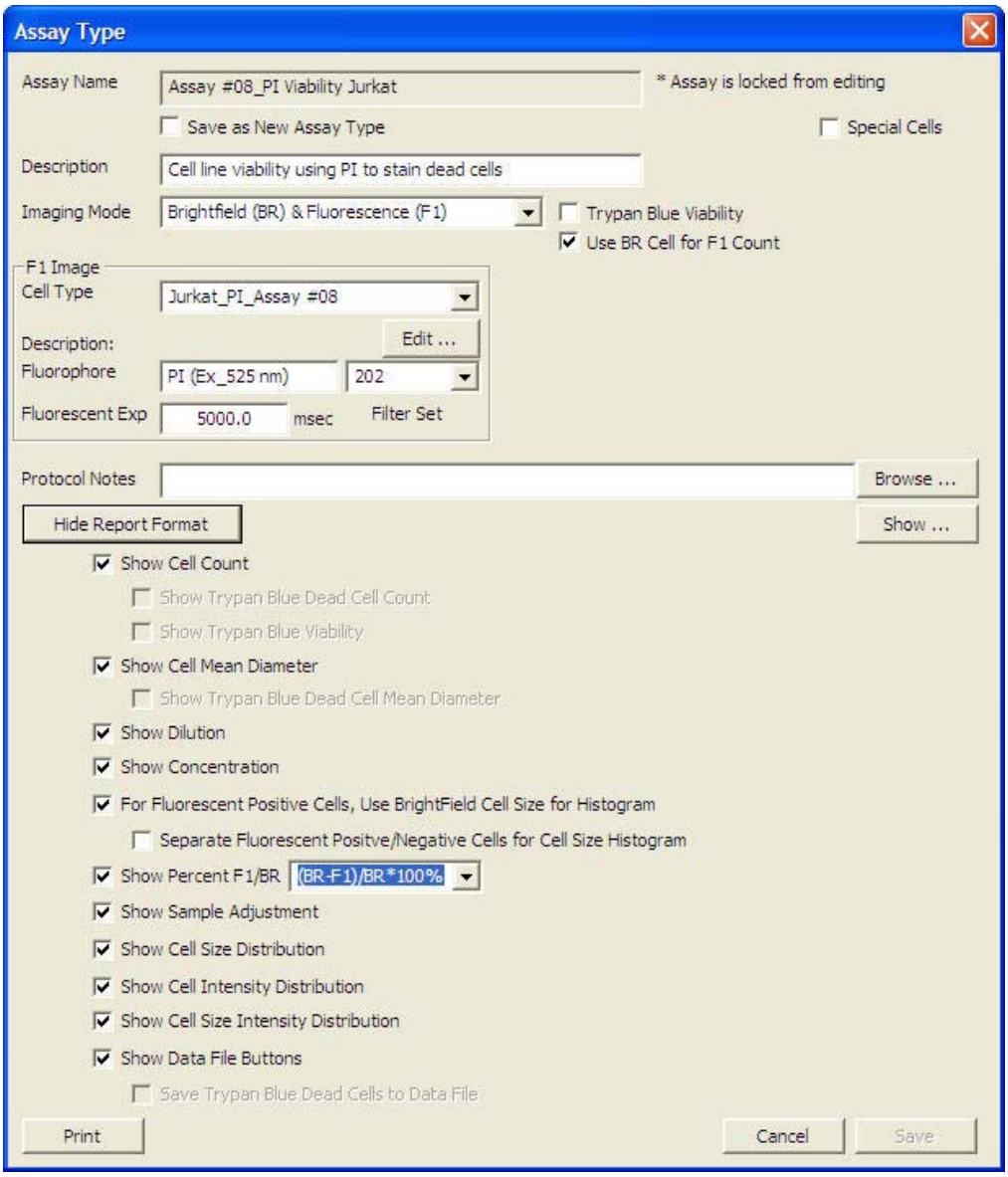

Step 4. Click '**Preview Brightfield Image**'

Step 5. Adjust focus if necessary

Step 6. Click **'Count'**

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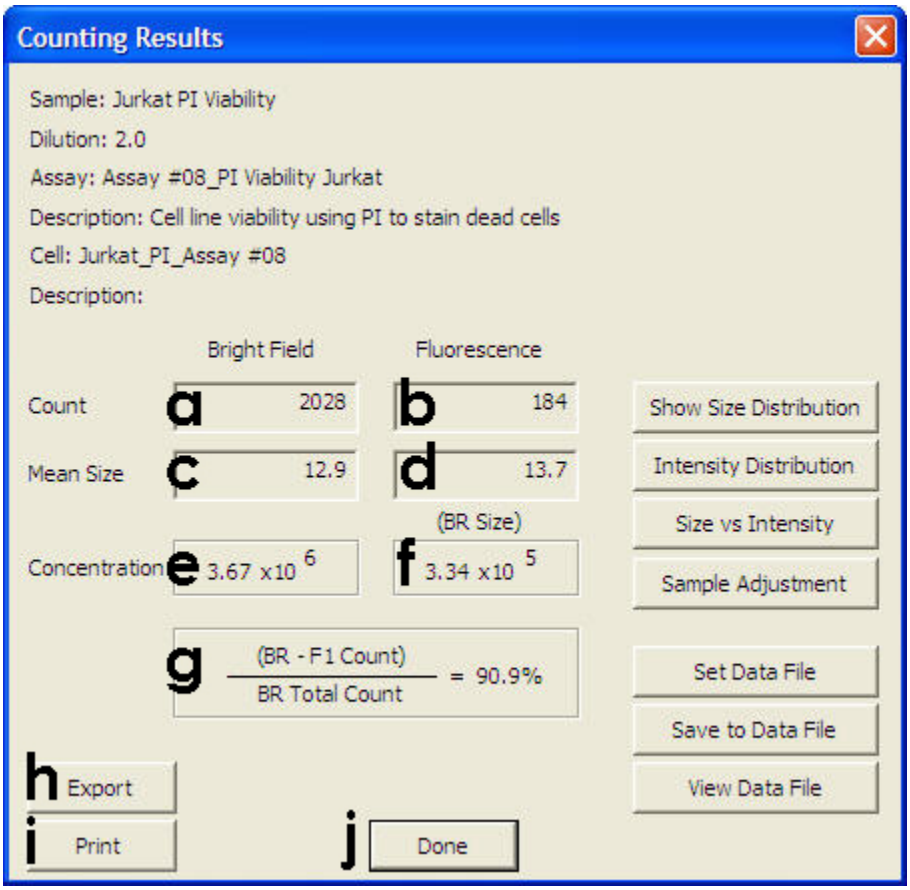
Step 7. Review **Counting Results** and display data as needed:



a. Count / Bright Field: *total number of cells counted in the brightfield image*
b. Count / Fluorescence: *number of fluorescence positive cells counted*
c. Mean Size / Bright Field: *mean diameter of cells measured in brightfield*
d. Mean Size / Fluorescence: *mean diameter of fluorescence positive cells*
e. Concentration / Bright Field: *total cell concentration (/mL) of original sample*
f. Concentration / Fluorescence: *fluorescence cell concentration (/mL)*
g. (BR-F1 Count) / BR Total Count: *% viability of cell sample*
h. Export: *stores all raw data, assay parameters and graphs in an Excel file*
i. Print: *send current counting results to printer*
j. Done: *closes counting results window*
*Note: After clicking '**Done**' the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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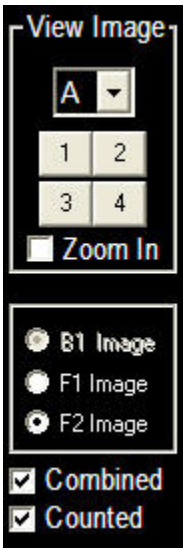
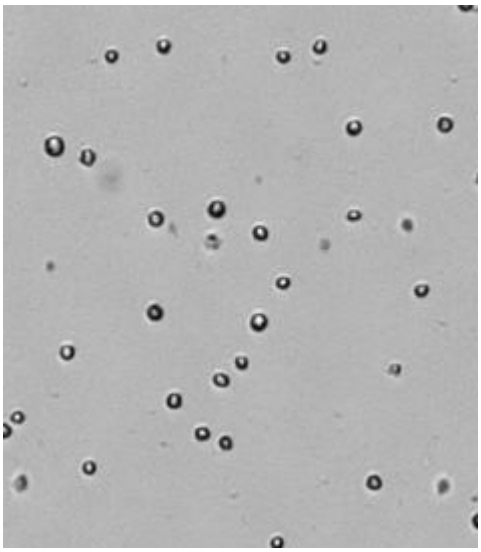
Step 8. Review Images on-screen

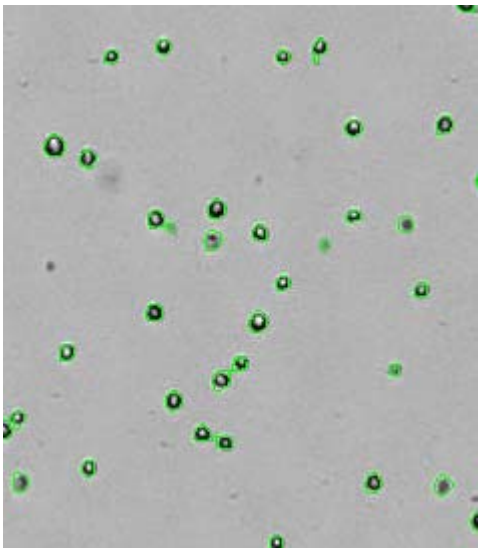


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Brightfield cell image



B. Counted cell image. Green outline indicates counted cells

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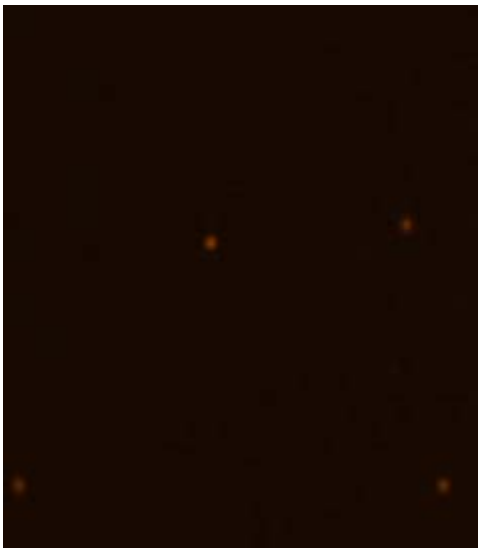
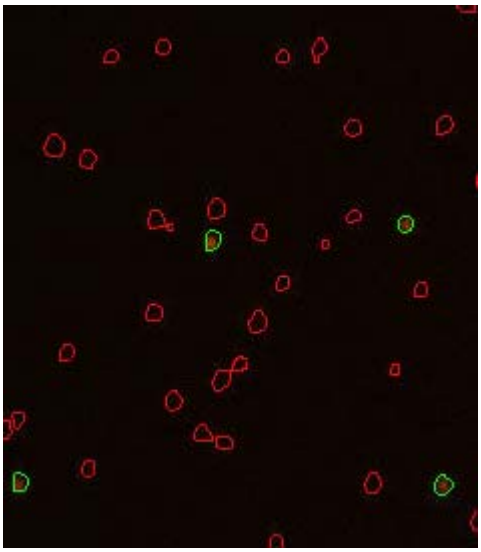


C. PI positive fluorescence cell image



D. Counted fluorescence cell image. Green outline indicates PI positive cells.

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Assay #09 Live Cell Concentration using Calcein AM

Calcein AM is used to determine cell viability and other key functional activities of cells. Calcein AM is non-fluorescent but when taken up by live cells, it is converted to calcein, a compound that emits in the ‘ green’ fluorescent range. After taking ‘green’ fluorescent images, all fluorescent cells are counted and their concentration in the sample is determined. Brightfield images can also be taken and counted. However, in the sample images for this assay, brightfield counting picked up debris that was eliminated by imaging in the fluorescent channel.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Mix cell samples with Caiceim AM and incubate according to manufacturer’s instructions.

Step 2. Load 20µL cell sample in one side of the cell counting chamber and insert into instrument.

Step 3. Select **Assay #09_Calcein AM_Macrophage** from the **Assay SETUP** drop-down menu:

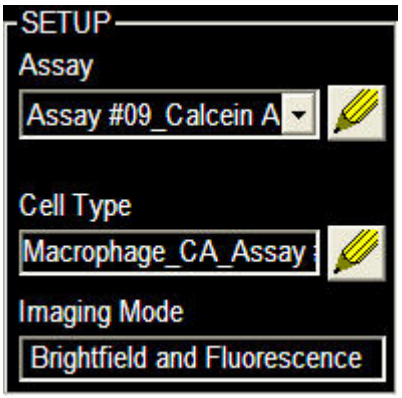
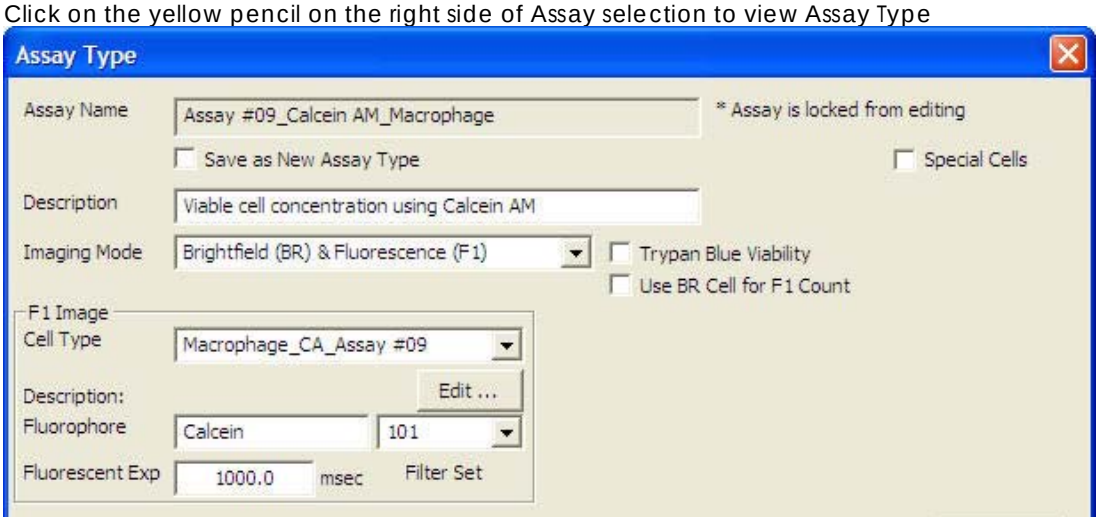


Image mode: Brightfield and Fluorescence

Click on the yellow pencil on the right side of Assay selection to view Assay Type



Step 4. Click **'Preview Brightfield Image'**

Step 5. Adjust focus if necessary

Step 6. Click **'Preview F1 Image'**

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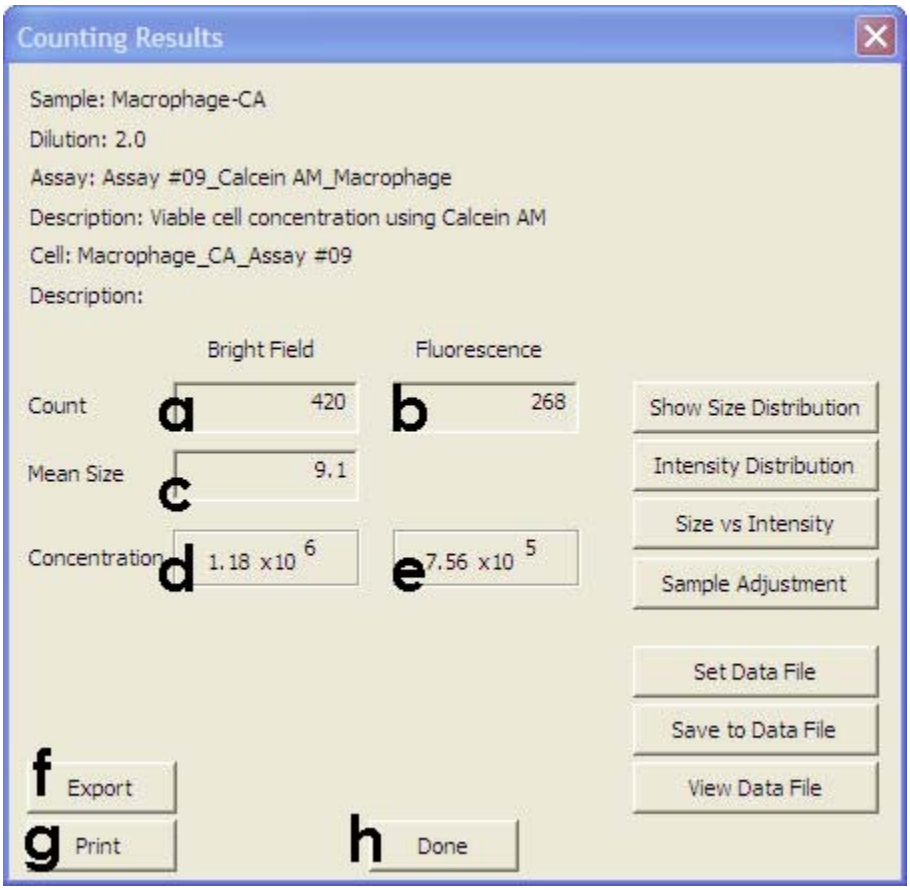
Step 7. Click **'Count'**

Step 8. Review **Counting Results** and display data as needed:



a. Count / Bright Field: *total number of cells counted in the brightfield image*
b. Count / Fluorescence: *number of fluorescence positive cells counted*
c. Mean Size / Bright Field: *mean diameter of cells measured in brightfield*
d. Concentration / Bright Field: *total cell concentration (/mL)*
e. Concentration / Fluorescence: *fluorescence cell concentration (/mL)*
f. Export: *stores all raw data, assay parameters and graphs in an Excel file*
g. Print: *send current counting results to printer*
h. Done: *closes counting results window*
*Note: After clicking '**Done**' the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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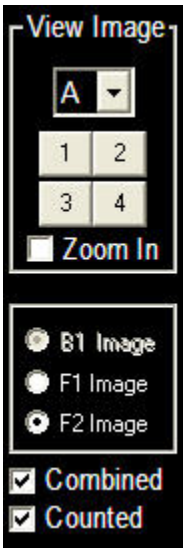
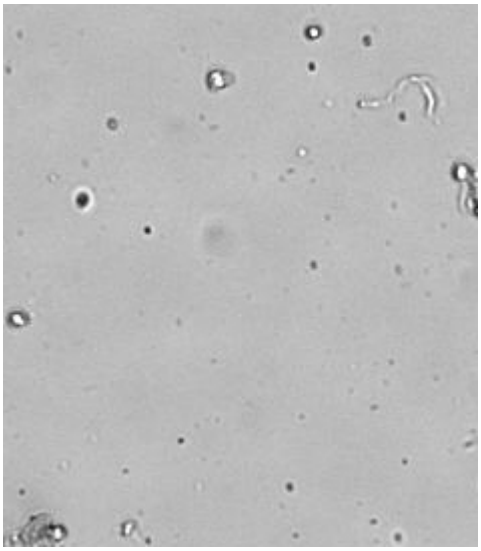
Step 9. Review Images on-screen

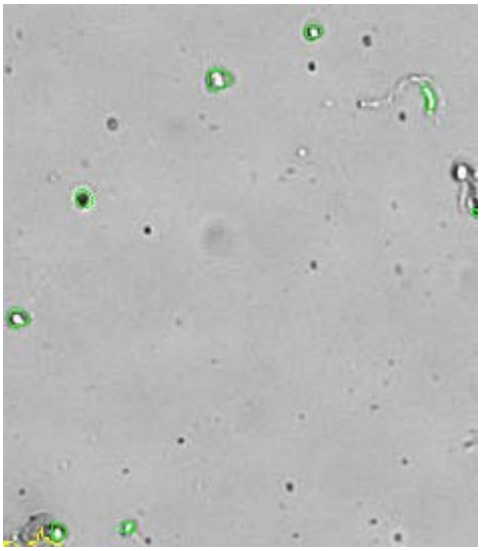


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Brightfield image of cells.



B. Brightfield after counting. This cell sample contains a large amount of debris which makes the brightfield counting method less accurate.

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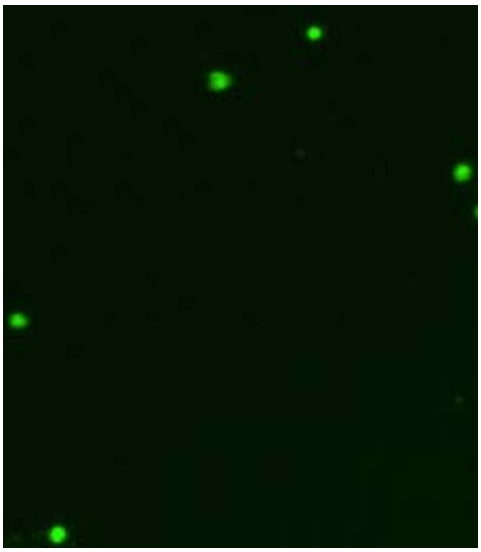
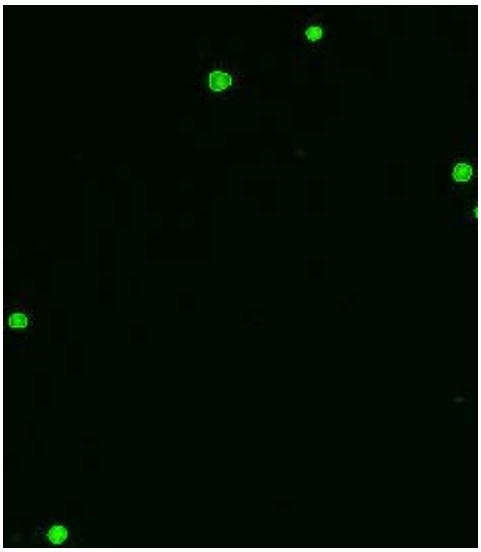


C. Fluorescence image of Calcein positive cells .



D. Fluorescence positive cells after counting

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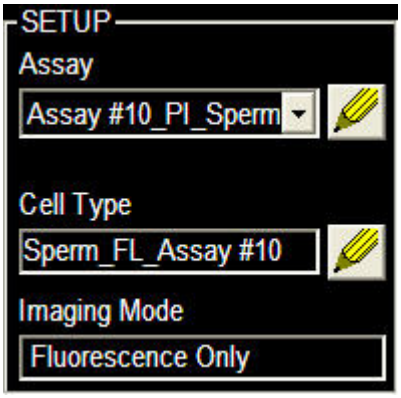
Assay #10 Sperm Cell Concentration Using Propidium Iodide Staining

In this assay, propidium iodide staining is used to count all cells in a sperm sample. PI is a fluorescent stain that emits in the 'red' range and is used to mark all sperm after permeabilization. After taking 'red' fluorescent images, all fluorescent cells are counted and their concentration in the sample is determined. This assay demonstrates that the Cellometer software can be used to count non-circular objects such as sperm.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

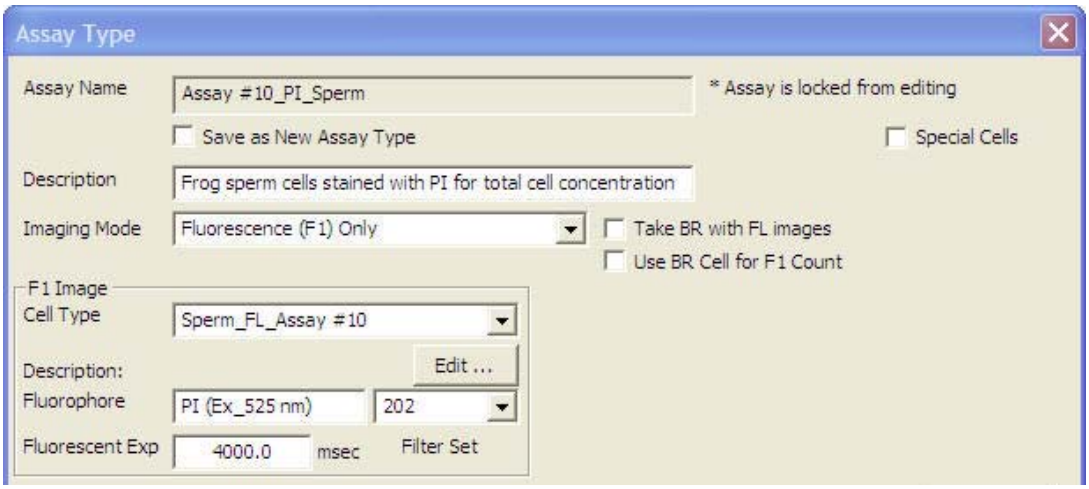
Step 1. Stain sperm cells with propidium iodide following manufacturer's instructions

Step 2. Select **Assay #10_PI_Sperm** from the **Assay SETUP** drop-down menu:



Imaging mode: Fluorescence Only

Click on the yellow pencil on the right side of Assay selection to view Assay Type



The Fluorescent Exposure time can be adjusted to obtain adequate signal within a range of PI concentrations since the signal strength is cell-dependent.

Step 4. Click '**Preview Brightfield Image**':

Step 5. Adjust focus if necessary

Step 6. Click **'Count'**

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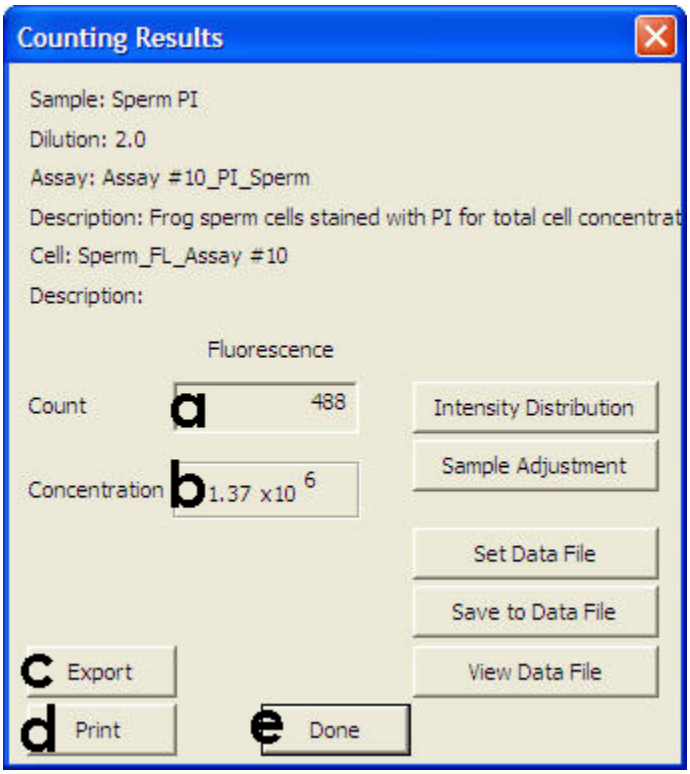
Step 7. Review **Counting Results** and display data as needed:



a. Count / Fluorescence: *number of fluorescence positive cells counted*
b. Concentration / Fluorescence: *fluorescence cell concentration (/mL)*
c. Export: *stores all raw data, assay parameters and graphs in an Excel file*
d. Print: *send current counting results to printer*
e. Done: *closes counting results window*
Note: After clicking **'Done'** *the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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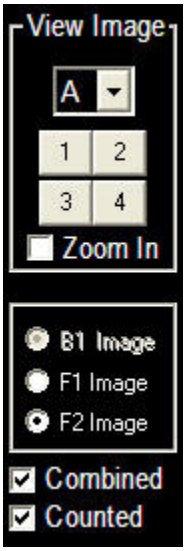
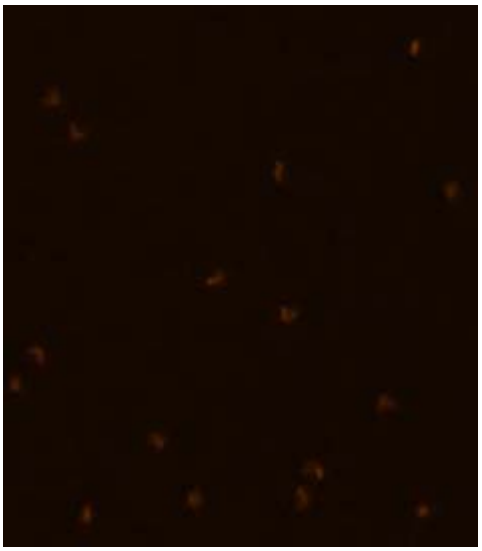
Step 8. Review Images on-screen

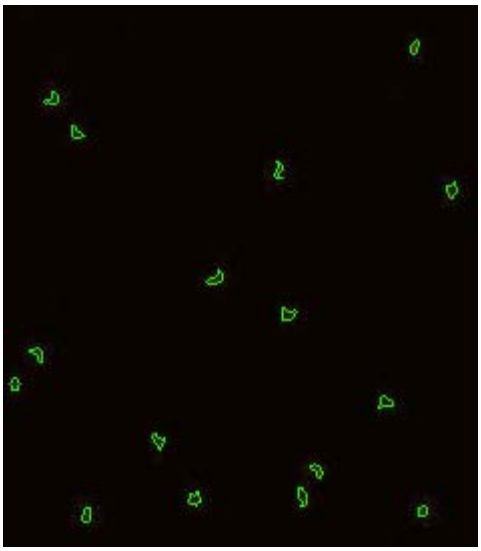


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Cell image before counting



B. Cell image after counting. Green outline indicates sperm cells counted

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Assay #11 Splenocyte Concentration and Viability Using AO/PI

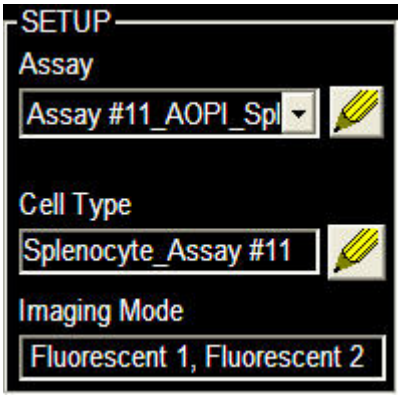
Acridine orange is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide is a fluorescent stain that only penetrates dead cells and emits in the 'red' range. After taking both ' green' and 'red' fluorescent images, all fluorescent cells in each channel are counted and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Mix 10 µl splenocyte sample with 10 µl AOPI mixture

Step 2. Load 20 µl in one cell counting chamber and insert into instrument

Step 3. Select **Assay #11_AOPI_Splenocyte** from the **Assay SETUP** drop-down menu:



Cell Type: Splenocyte_Assay #11 is displayed
Imaging Mode: Fluorescent 1, Fluorescent 2

The fluorescent 1 is used to measure live cells, while the fluorescence 2 is used to measure dead cells.

Both Cell Type and Imaging Mode are defined in the Assay parameters, and can be shown from the main screen by clicking on the pencil icon.

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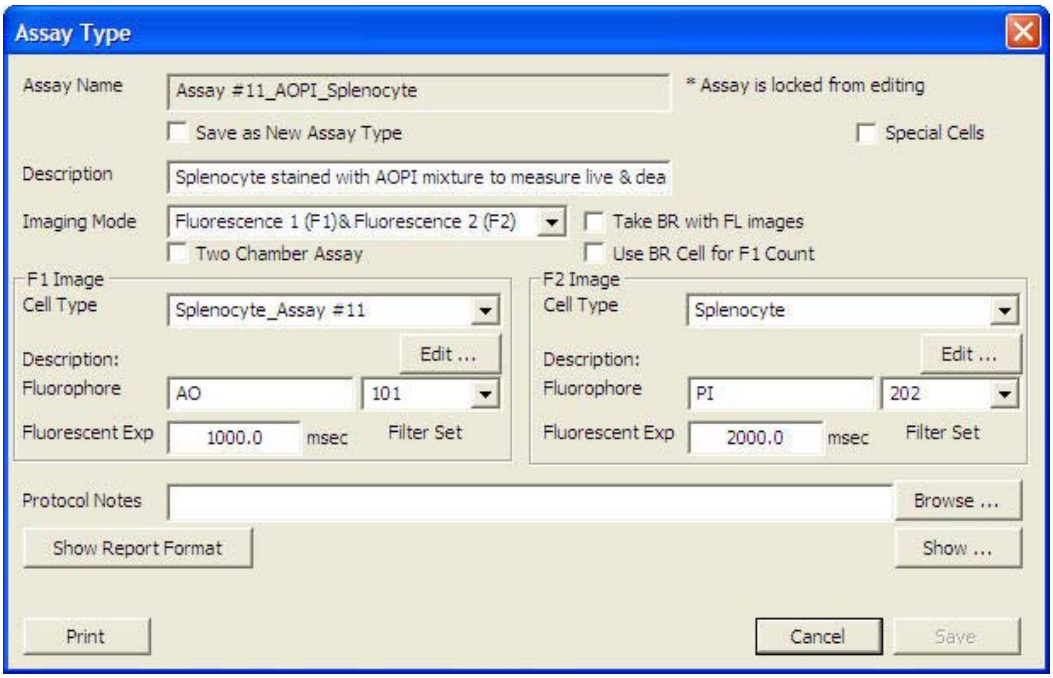
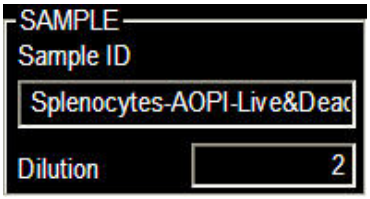
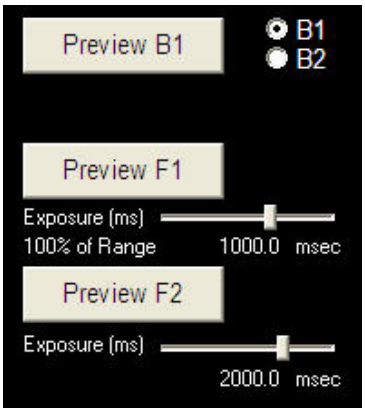


Step 4. Input Sample ID and dilution factor



Step 5. Input "2" for Dilution

Step 6. Review cell images



a. Click **'B1'**
b. **'Preview B1'** will display brightfield image of F1 channel
c. Adjust Focus if necessary
d. Stop Review
e. Click **'Preview F1'** (Fluorescence)
f. 100% of Range indicates adequate exposure time for F1 channel
g. Click **'Preview F2'** (Fluorescence)

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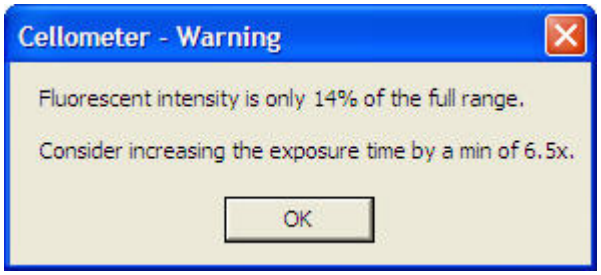
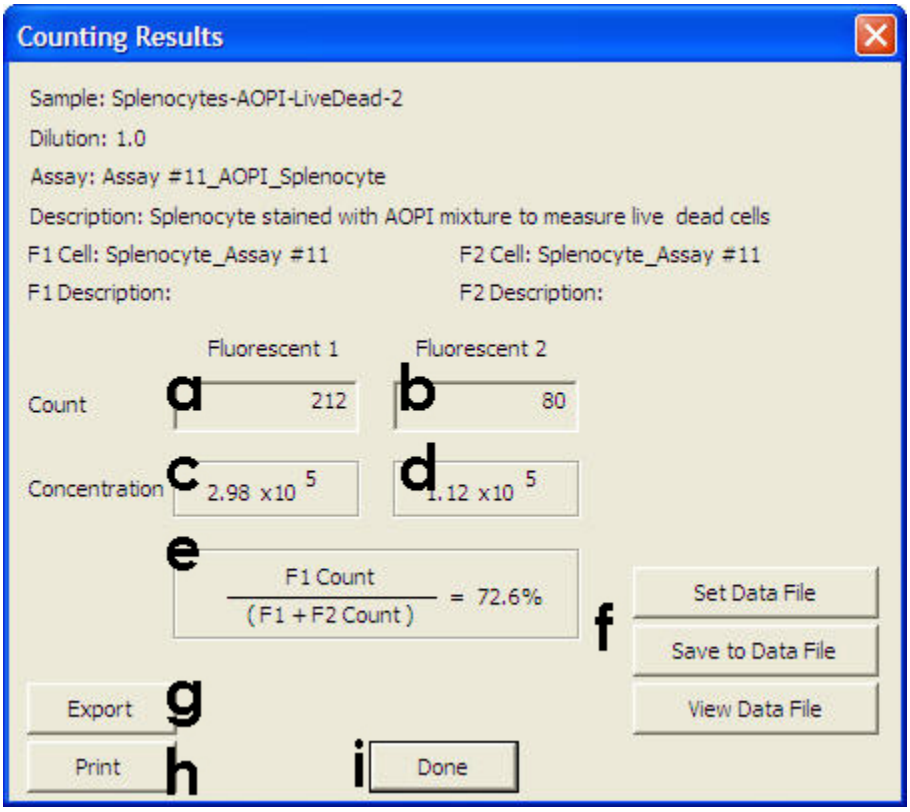

Cellometer - Warning
Fluorescent intensity is only 14% of the full range.
Consider increasing the exposure time by a min of 6.5x.
OK

h. If exposure time is inadequate, increase exposure time by using the pencil tool next to the Assay Set up drop down menu

Step 7. Review **Counting Results** and display data as needed:



a. Count / Fluorescent 1: *indicates total number of live cells*
b. Count / Fluorescent 2: *indicates total number of dead cells*
c. Concentration/ Fluorescent 1: *indicates live cell concentration /(mL)*
d. Concentration/ Fluorescent 2: *indicates dead cell concentration /(mL)*
e. F1 Count /(F1 +F2Count): *indicates percentage of live cells to total cells*

f. Save to Data File: *record counting results in a table*
g. Export *stores all raw data, assay parameters and graphs in an Excel file*
h. Print: *send the current Counting Results to a printer*
i. Done: *closes counting results window*
Note: After clicking **'Done'** *the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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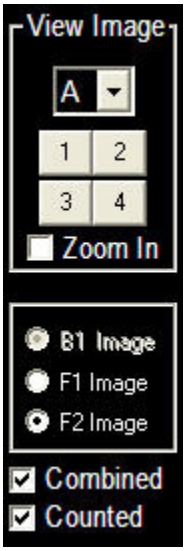
Step 8. Review Images on-screen



A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.

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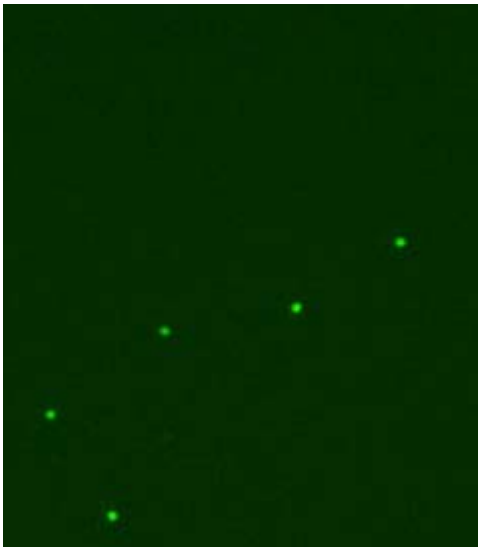
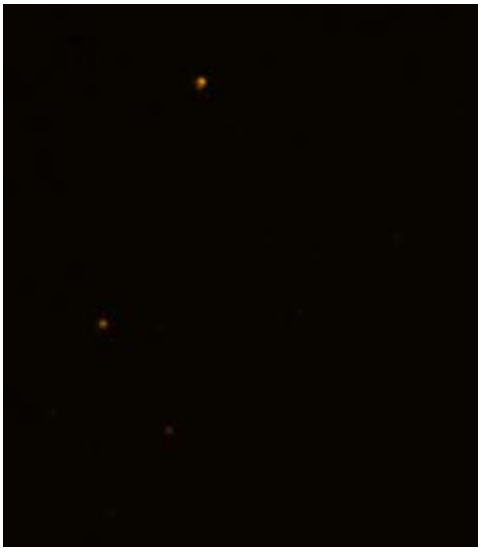
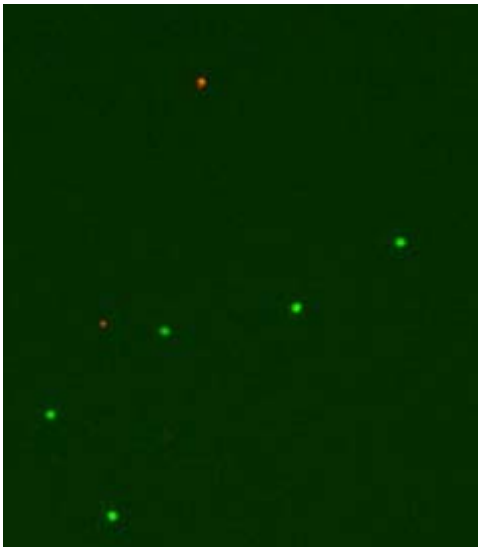


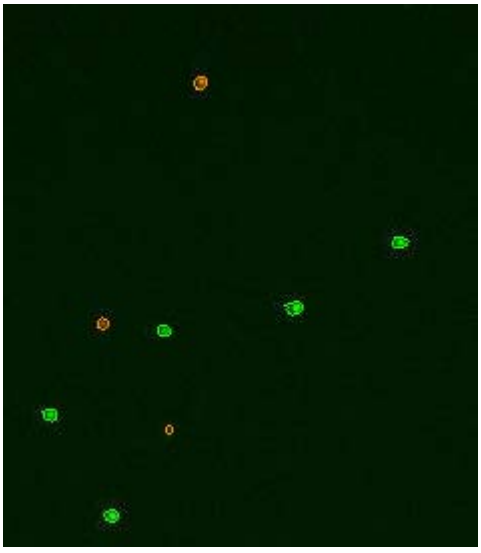
A. F1



B. F2



C. Combined



D. Combined and Counted

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Assay #12 Splenocyte Concentration and Viability Using AO/EB

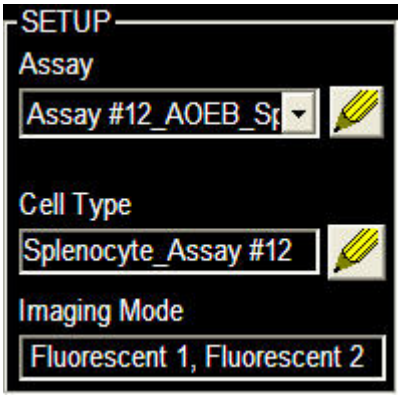
Acridine orange is a nuclear stain that emits in the 'green' range and is used to stain live cells. Ethidium bromide is a fluorescent stain that only penetrates dead cells and emits in the 'red' range. After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.

Note: *Assay parameters for assays that are included in the software are locked and can not be edited. To customize this assay to suit your particular application, please see* ***Modify Default Assay Parameters*** *for instructions on how to modify and save as a new assay.*

Step 1. Mix 10 µl splenocyte sample with 10 µl AOEB mixture

Step 2. Load 20 µl in one cell counting chamber and insert into instrument

Step 3. Select **Assay #12_AOEB_Splenocyte** from the **Assay SETUP** drop-down menu:



Cell Type: Splenocyte_Assay #12 is displayed
Imaging Mode: Fluorescent 1, Fluorescent 2

The fluorescent 1 is used to measure live cells, while the fluorescence 2 is used to measure dead cells.

Both Cell Type and Imaging Mode are defined in the Assay parameters, and can be shown from the main screen by clicking on the pencil icon.

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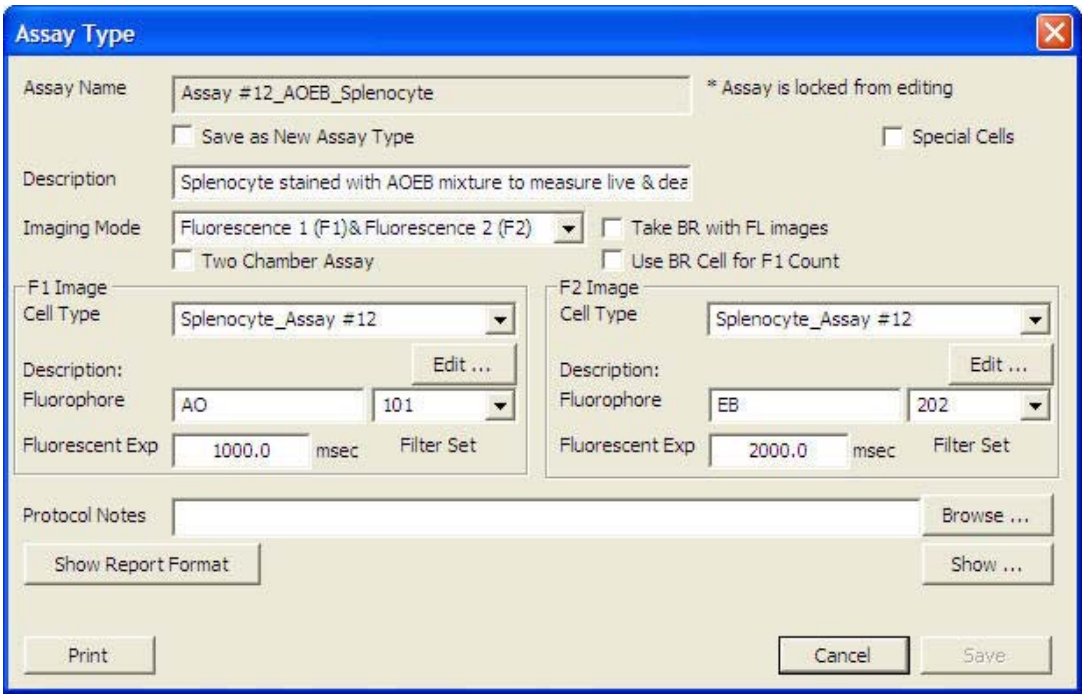
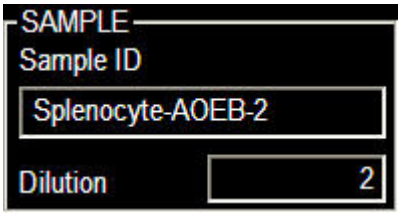


Step 4. Input Sample ID and dilution factor



Step 5. Input "2" for Dilution

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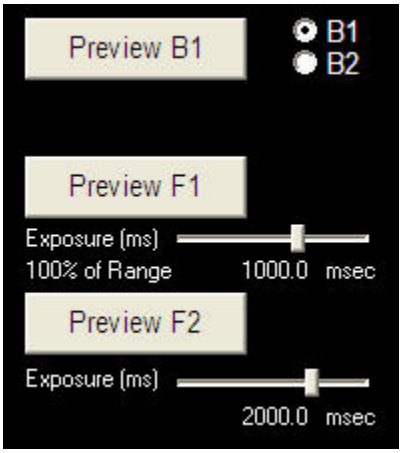
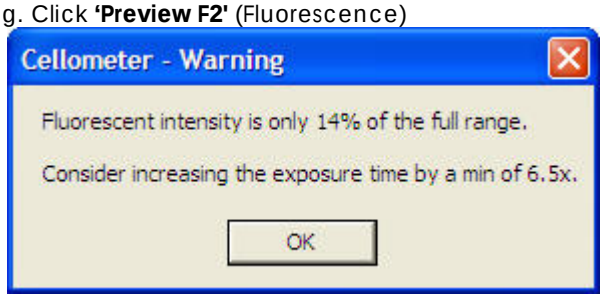
Step 6. Review cell images



a. Click **'B1'**
b. **'Preview B1'** will display brightfield image of F1 channel
c. Adjust Focus if necessary
d. Stop Review
e. Click **'Preview F1'** (Fluorescence)
f. 100% of Range indicates adequate exposure time for F1 channel
g. Click **'Preview F2'** (Fluorescence)


Cellometer - Warning
Fluorescent intensity is only 14% of the full range.
Consider increasing the exposure time by a min of 6.5x.
OK

h. If exposure time is inadequate, increase exposure time by using the pencil tool next to the Assay Set up drop down menu

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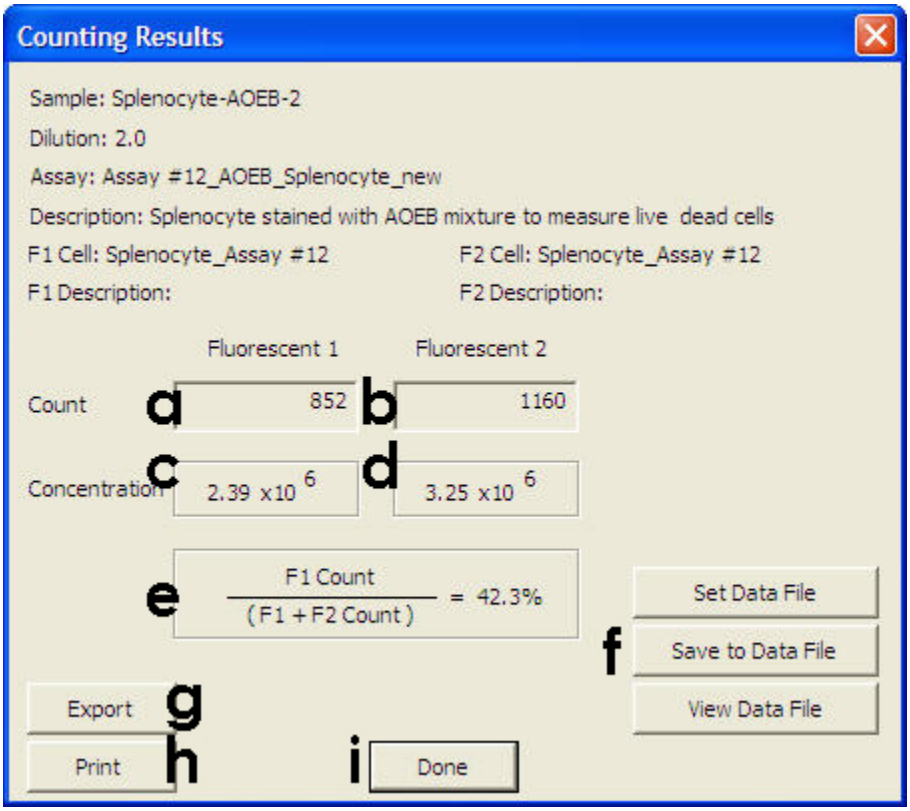
Step 7. Review **Counting Results** and display data as needed:



a. Count / Fluorescent 1: *indicates total number of live cells*
b. Count / Fluorescent 2: *indicates total number of dead cells*
c. Concentration/ Fluorescent 1: *indicates live cell concentration /(mL)*
d. Concentration/ Fluorescent 2: *indicates dead cell concentration /(mL)*
e. F1 Count /(F1 +F2Count): *indicates percentage of live cells to total cells*

f. Save to Data File: *record counting results in a table*
g. Export: *stores all raw data, assay parameters and graphs in an Excel file*
h. Print: *send the current Counting Results to a printer*
i. Done: *closes counting results window*
Note: After clicking **'Done'** *the counting results box can be redisplayed at any time by clicking on the 'View Report of Counting Results' button*:

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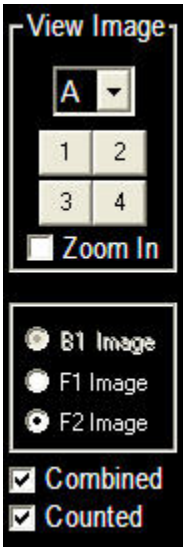
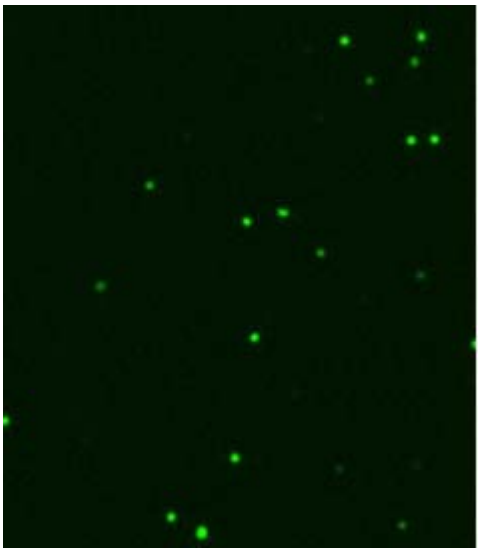
Step 8. Review Images on-screen

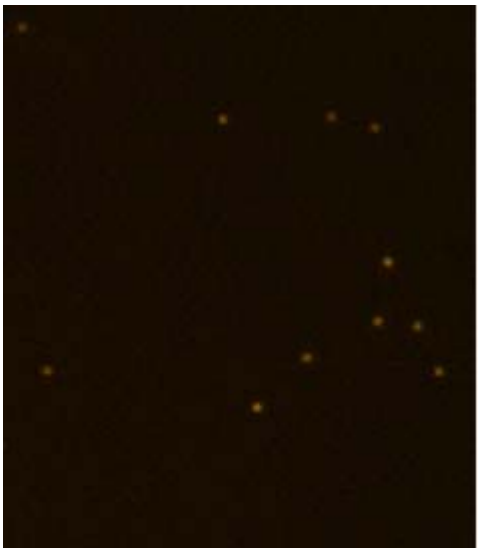


A: one of the four captured images
1, 2, 3 or 4: one quadrant of a selected image
F1: fluorescence 1 image view
F2: fluorescence 2 image view
Combined: check to display an overlay of F1 and F1 images
Counted: check to see counted cells outlined

When both fluorescence channels are used check the **Combined** box to display an overlay of F1 and F2 captured images.



A. Fl



B. F2

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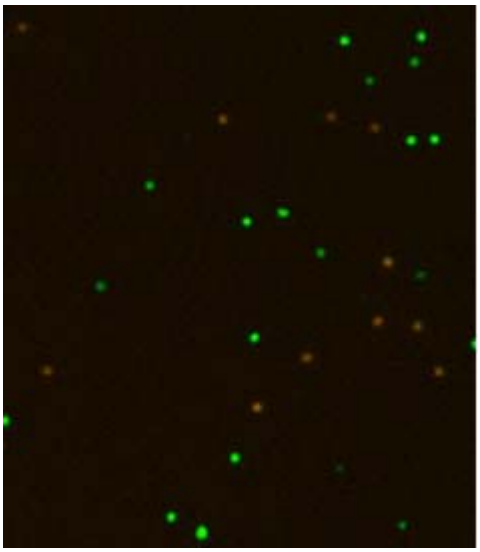
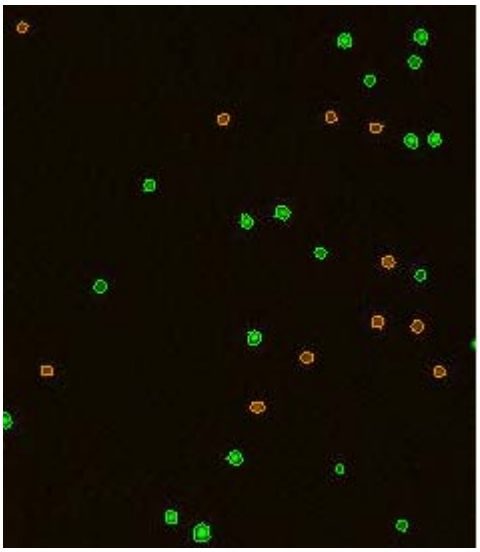


C. Combined



D. Combined and Counted

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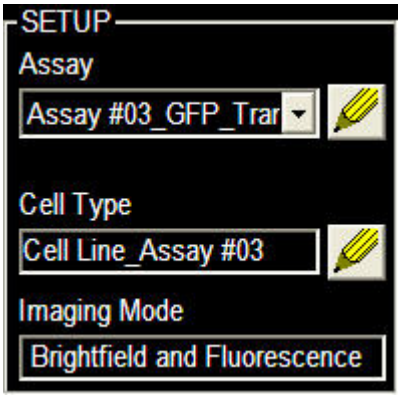
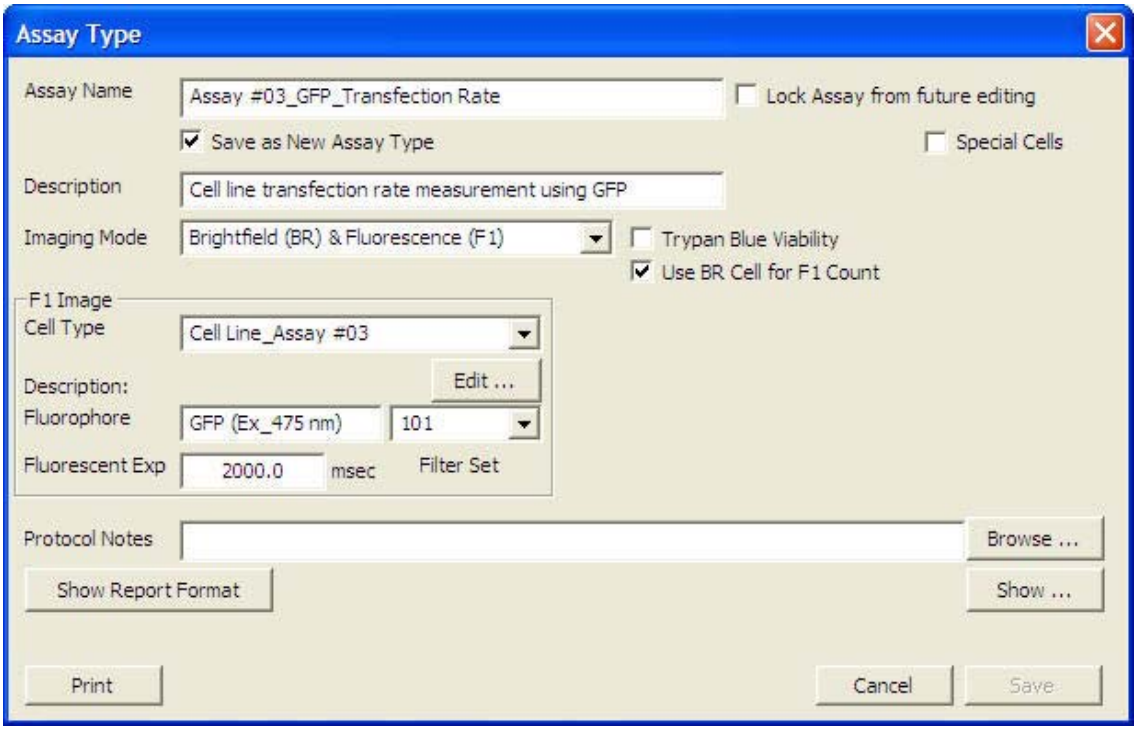
Modify Default Assay Parameters

Vision default assay types are intended as guidelines for setting up new assays. The following is a procedure to modify an existing assay type to suit specific experiments.

Step 1. Select **Assay #03_GFP_Transfection Rate** from **Assay SETUP** drop-down menu:



Step 2. Edit Assay Type by click on the yellow pencil tool



a. Check **Save as New Assay Type**
b. Input new or modified name in **Assay Name** (**Save** will turn on after the assay name is changed.)
c. Modify cell type if necessary
   Method 1: drop-down from **F1 Image Cell Type** to find another cell type
   Method 2. Edit to change current cell type parameters

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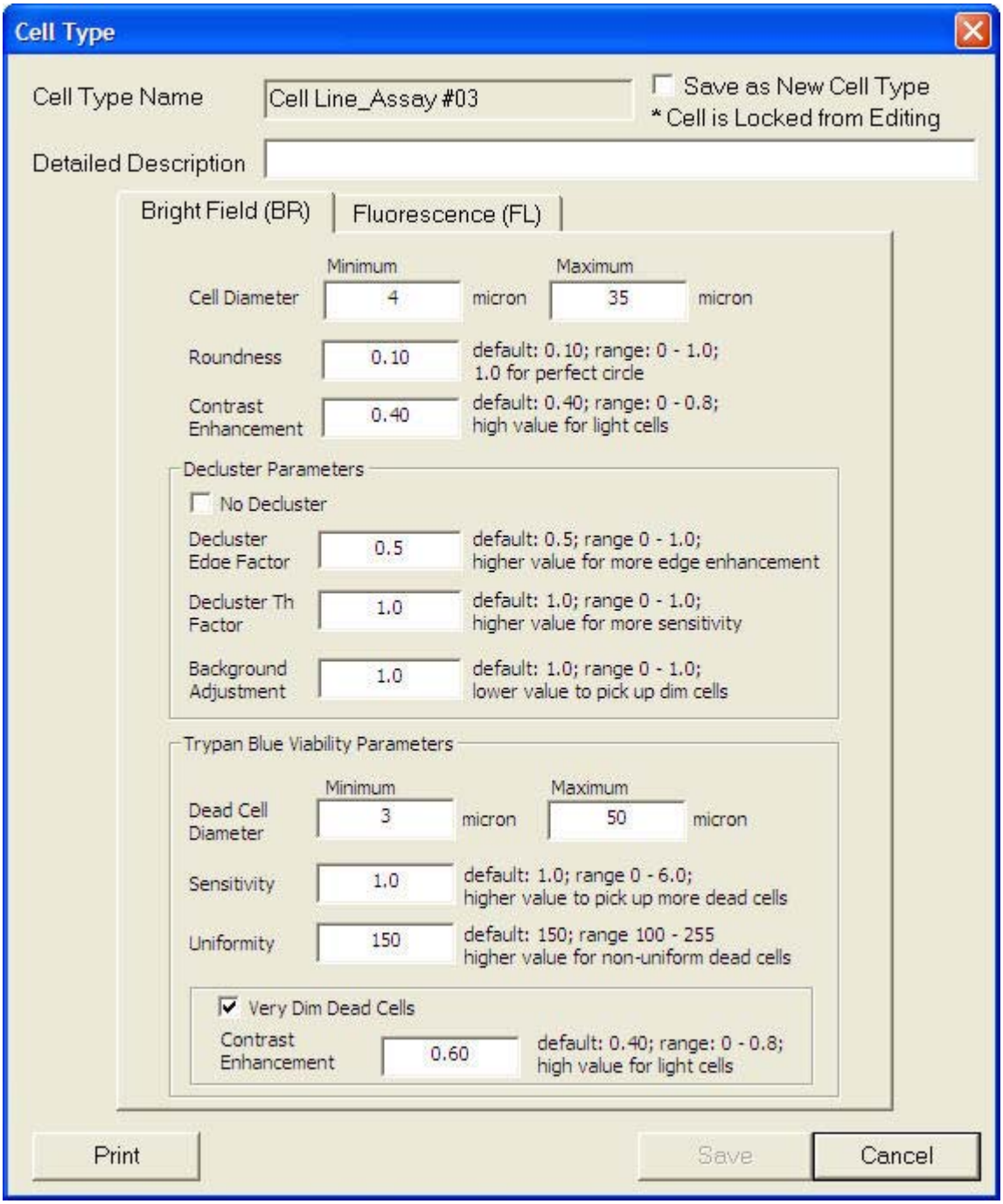

Check **Save as New Cell Type**
Change Cell Type Name
Input modification to the cell type parameters
Save

d. Change Fluorescence exposure time if needed

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IV

Operation Reference

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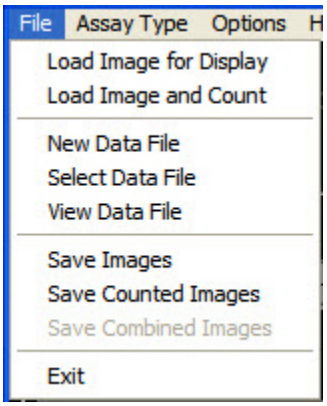
Software Operation Overview

The following section describes each function available in the software main menu.

File Menu:



File > Load Image for Display
Load saved cell images for display. When Assay Type includes both brightfield and fluorescence, multiple images are required.

File > Load Image and Count
Load saved cell images for counting. When Assay Type includes both brightfield and fluorescence, multiple images are required. Saved cell images are counted using current Assay Type parameters.

File > New Data File
Start a new text file for Cell Count Table

File > Select Data File
Select a different text file for saving Cell Count Table

File > View Data File
Display currently selected Cell Count Table

File > Save Images
Save cell images

File > Save Counted Images
Save cell images with outlines indicating counted objects

File > Save Combined Images
Save the combined view showing F1 and F2 fluorescent objects in a single merged image

File > Exit
Quit Cellometer Vision software

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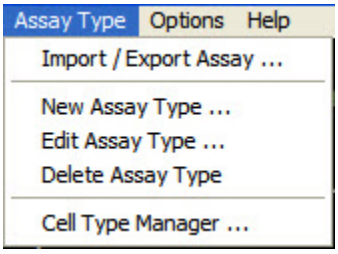
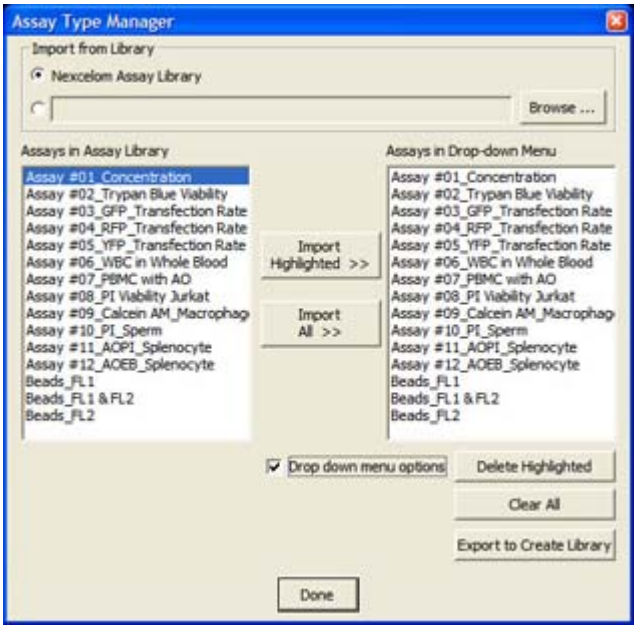
Assay Type Menu:

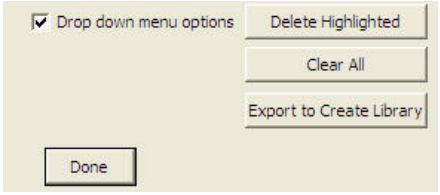


Assay Type > Import /Export Assay

The Assay Type Library is a list of defined assay types. Assay Type Manager is used to import from the existing assay library or to export a user defined list of assay types into a new Assay Library:



Nexcelom Assay Library: *default library from Nexcelom*
Browse: *select location for user to save Assay Library*
Assay in Assay Library: *list of Assay Types in an Assay Library*
Assays in Drop-down Menu: *List of Assays in the Vision software Setup Assay Drop-down list*
Import Highlighted: *Import highlighted assay type in a selected assay library to the Assay drop-down menu in Setup*
Import All: *import the complete list of Assays from the Assay Library to the drop down menu*
Drop down menu options: *de-select to hide delete and export functions:*



Delete Highlighted: *delete highlighted assay type*
Clear All: *clear all assay types in the drop down menu*
Export to Create Library: *export all the assay types in the drop down menu to a new Assay Library*
Done: *close Assay Manager*

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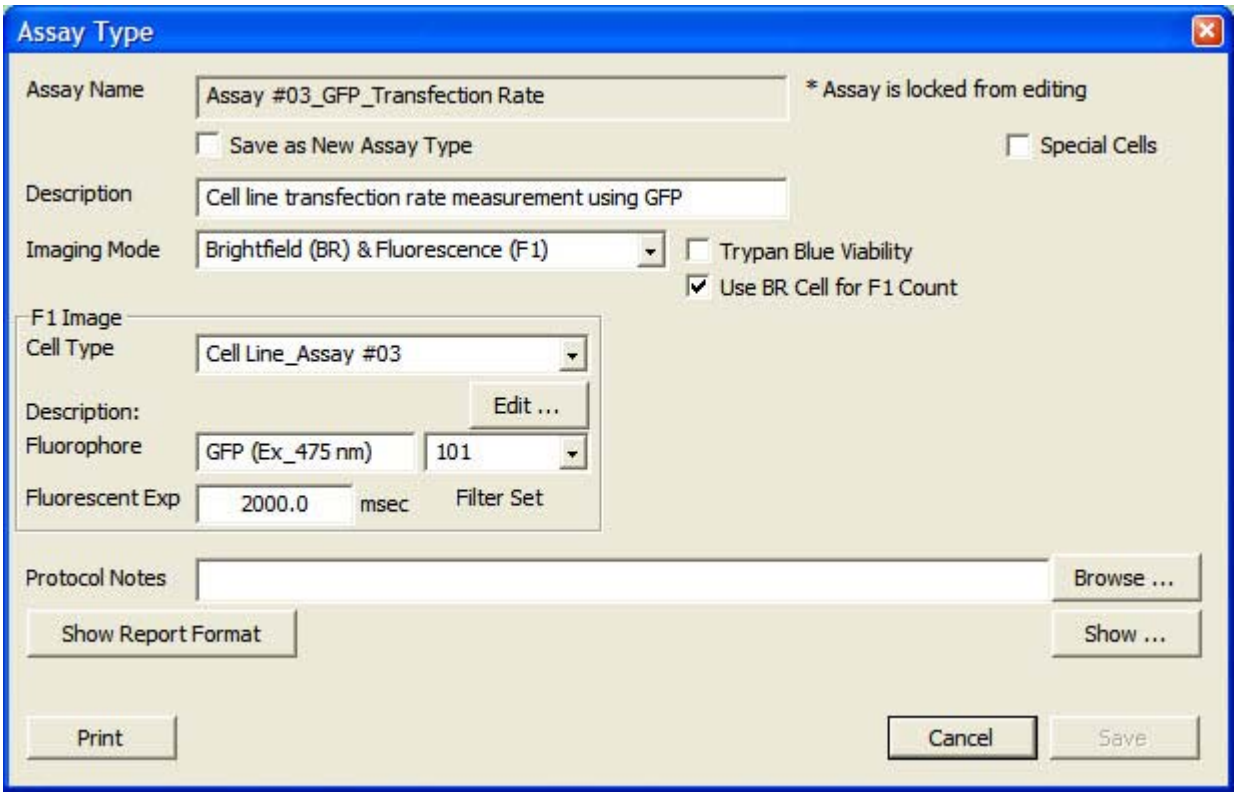
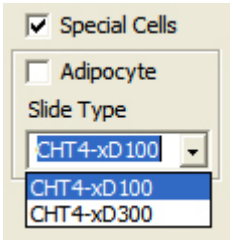
Assay Type -> Edit Assay Type:



Assay Name: *name of assay that appears in the Assay drop down list*
Description: *text descriptions for your assay*
Save as New Assay Type: *check to save current Assay Type with a different name. User can then edit parameters for the new Assay Type*
Lock Assay from future editing: *check to prevent future editing. Parameters cannot be changed later. Default assays are locked in software upon install.*
Special Cells check box:



Check 'Adipocyte' to select PD300 counting chambers with larger thickness

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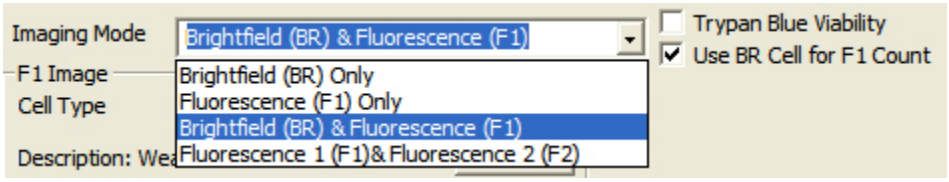
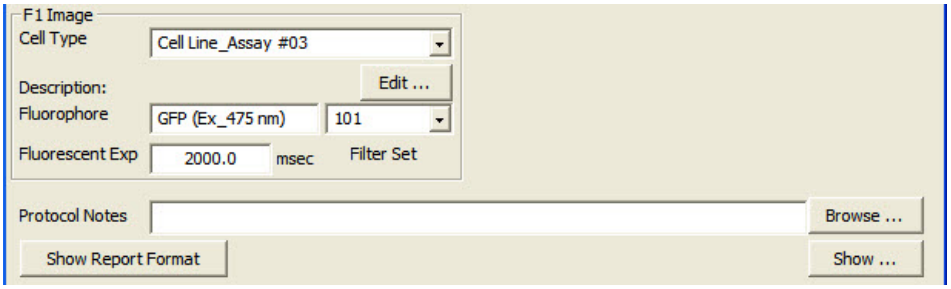
Imaging Mode:



Brightfield (BR) Only: *uses only brightfield to count cells and measure size*
Fluorescence (F1) only: *use only fluorescence to count cells*
Brightfield (BR) and Fluorescence (F1): *acquire both brightfield and fluorescence images. Cell counting results are produced using a combination of both images*
Fluorescence 1(F1) and Fluorescence 2(F2): *use two different fluorescence images to produce counting results*
Trypan Blue Viability: *Check for trypan blue viability*
Use BR Cell for F1 Count: *Measure Fluorescence intensity only within cells located in Brightfield (BR) image.*



F1 Image: *Contains Cell Type and Fluorescence parameters for the F1 image.*
F2 Image: *(Not shown) Visible in 'Fluorescence 1 and Fluorescence 2' Imaging Mode, contains Cell Type and Fluorescence parameters for the F2 image.*
Cell Type: *drop down menu to select cell parameters*
Edit: *edit selected cell type parameters*
Fluorophore: *User defined name of Fluorophore used in this assay (Note: 101 or 202 indicates the filter set that detects that particular fluorophore)*
Fluorescent Exp: *Fluorescence exposure time in milliseconds.*
Protocol Notes: *Link to any user defined file containing notes/information about this particular assay*
Show: *Opens file loaded in 'Protocol Notes'*
Browse: *find saved protocols*

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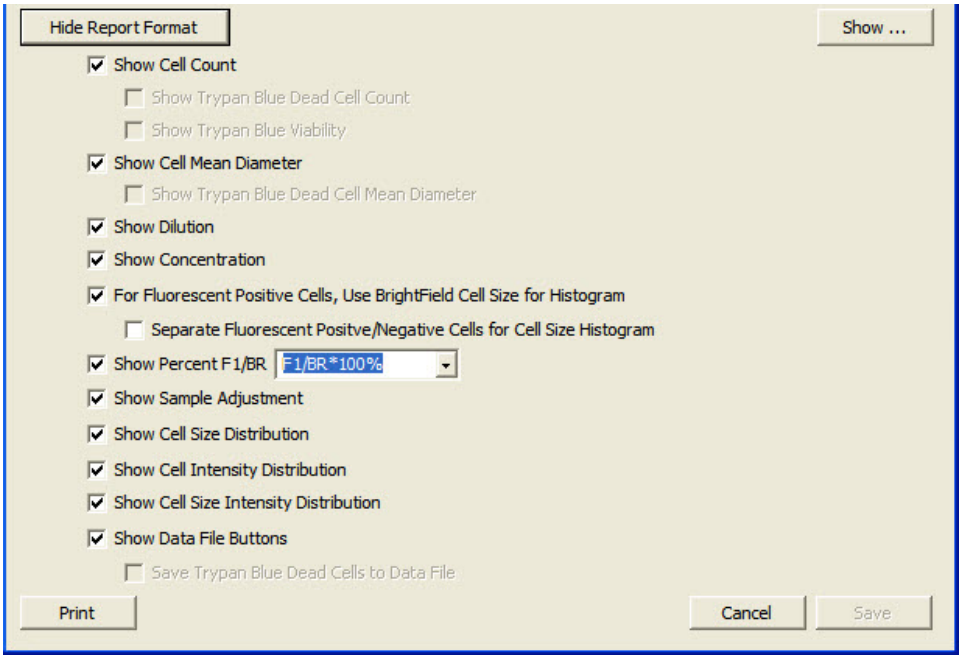
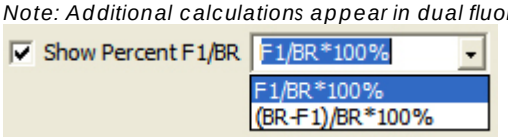


Show Report Format: *select to display Counting Results display option buttons that appear on the right hand side of the Counting Results box:*

The following options allow you to customize which results are displayed in the Counting Results dialog box:

Show Cell Count: *Check to display cell counts*
Show Trypan Blue Dead Cell Count: *Check to display dead cell counts*
Show Trypan Blue Viability: *Check to display the % viability*
Show Cell Mean Diameter: *Check to display the mean diameter of counted cells*
Show Trypan Blue Dead Cell Mean Diameter: *Check to display the mean diameter of trypan blue stained dead cells*
Show Dilution: *Check to display the dilution factor entered in the main window*
Show Concentration: *Check to display the cell concentration*
For Fluorescent Positive Cells, Use Brightfield Cell Size for Histogram: *Check to use the brightfield cell size when generating the cell size histogram for fluorescent cells*
Separate Fluorescent Positive/Negative Cells for Cell Size Histogram: *Check to generate a cell size histogram with fluorescent positive cells shown as green bars and fluorescent negative cells as red bars*
Show Percent F1/BR: *Indicates how is calculated. Ex: To show percentage of Fluorescent Positive cells, choose F1/BR*100%. To show percentage of Fluorescent Negative cells, choose (BR-F1)/BR*100%.*
Note: Additional calculations appear in dual fluorescence modes.



Show Sample Adjustment: *Check to show the **'Sample Adjustment'** button*
Show Cell Size Distribution: *Check to show the **'Show Size Distribution'** button*
Show Cell Intensity Distribution: *Check to show the **'Intensity Distribution'** button*
Show Cell Size Intensity Distribution: *Check to show the **'Size vs Intensity'** button*
Show Data File Buttons: *Check to show the **'Set Data File'**, **'Save to Data File'** and **'View Data File'** buttons*

Print: *print out selected Cell Type parameters*
Cancel: *close Assay Type without saving changes*
Save: *saves changes to options*

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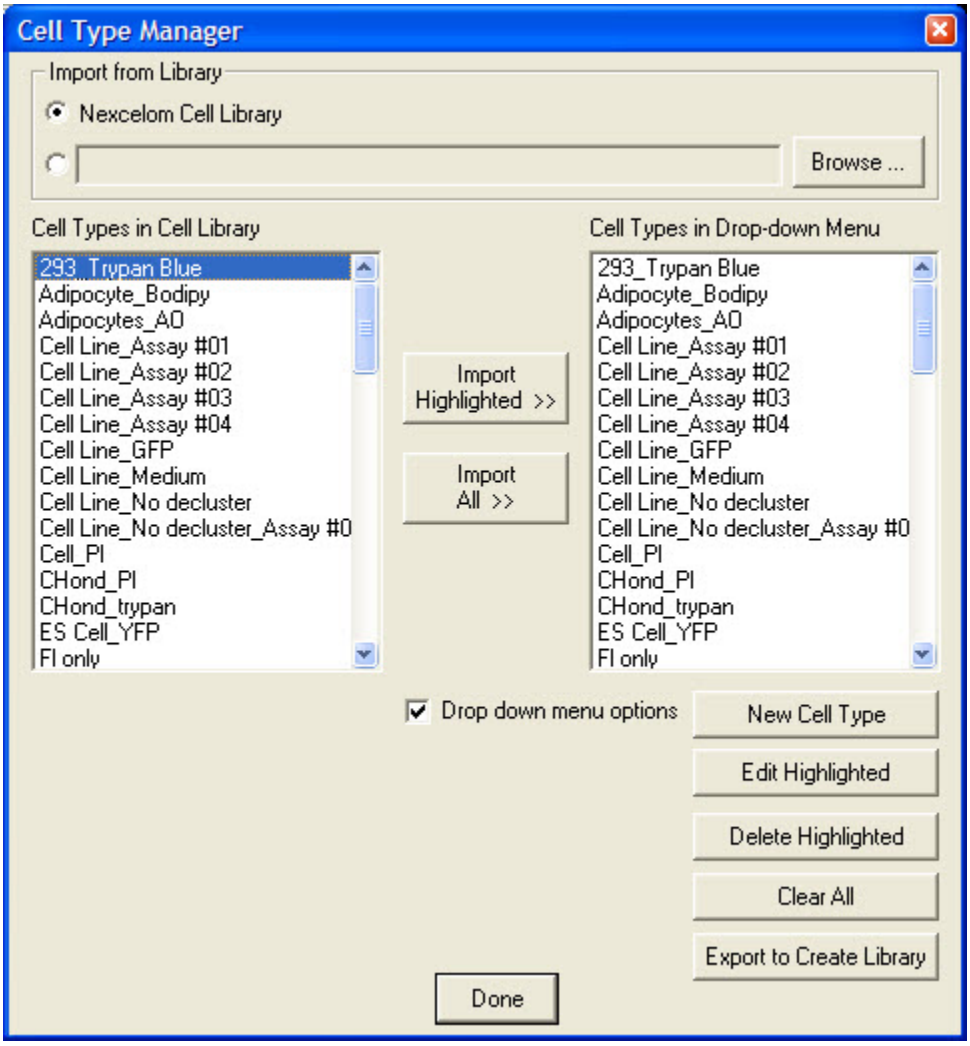
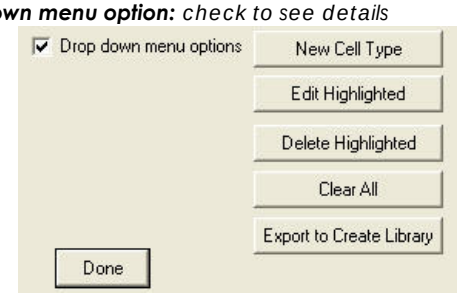
Assay Type > Delete Assay Type

Delete selected Assay from Setup Assay drop down list

Assay Type > Cell Type Manager



Nexcelom Cell Library: *built-in cell library from Nexcelom*
Browse: *locate user defined Cell library*
Cell Types in Cell Library: *list of Cell Types in a Cell Library*
Cell Types in Drop-down Menu: *list of Cell Types in the drop-down menu located in the Assay Type*
Import Highlighted: *import highlighted cell type from a Cell Library*
Import All: *import all cell types from the Cell Library*
Drop down menu option: *check to see details*



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New Cell Type: *start a new cell type from default setting*
Edit Highlighted: *edit the highlighted cell type*
Delete Highlighted: *delete highlighted cell type*
Clear All: *clear all cell types in the drop down list*
Export to Create Library: *generate a new Cell Type Library from the list of cell types*
Done: *close Cell Type Manager*

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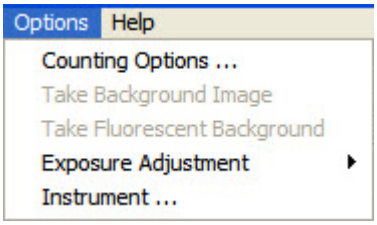
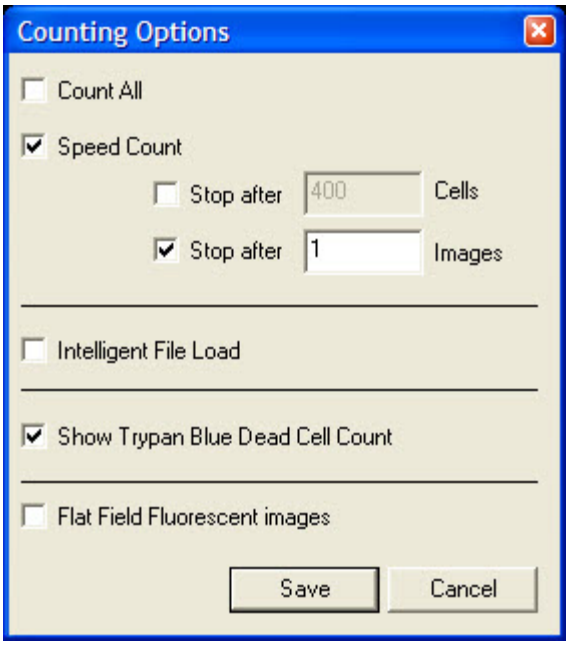
Options Menu:



Options > Counting Options



Count All: *check to count all four pre-defined locations inside each counting chamber*
Speed Count: *check for count without finishing all four pre-defined locations*
Stop after # of Cells: *check to stop counting after # of user defined cells are counted and the next frame has finished counting*
Stop after # of Images: *stop counting after finishing user defined images that are less than 4 images*
Show Trypan Blue Dead Cell Count: *check to display dead cell counts on the main software panel*
Use Background file with Fluorescence images: *allow fluorescence background image to be used for data analysis*
OK: *close Counting Option and save changes*
Cancel: *close Counting Option menu without saving changes*

Options > Take Background Image

Take brightfiled background image of the Vision system without cell counting chamber. For Vision Trio instruments, Background Images are needed for both fluorescence channels.

Options > Take Fluorescence Background

This function currently not available

Options > Exposure Adjustment

Show Exposure Adjustment: *display Exposure time*
Save Exposure Time as Default: *save an Exposure time that has been changed as default. When the Vision software is closed and restarted, the saved exposure time will be used*

Options -> Instrument

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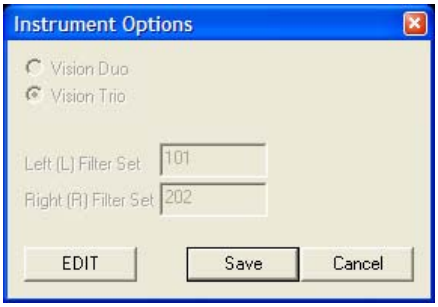

Note: *These are factory settings and are only editable when changing hardware configuration. Please contact Nexcelom for additional information.*
Vision Duo/Vision Trio: *Indicates which instrument the software is configured for (Factory Setting)*
Left (L) Filter Set/Right (R) Filter Set: *Displays Filter Sets installed in the machine (Vision Trio Only)*

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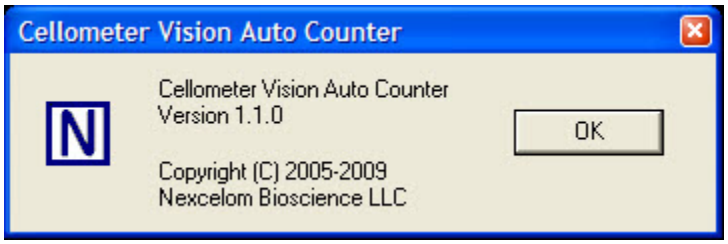
Help Menu:

Help> Vision Help: User manual and Tutorials

Help> About Nexcelom Vision (V1.1.0):

Displays version number of Cellometer Vision software currently installed



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V

Technical Information

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Specifications

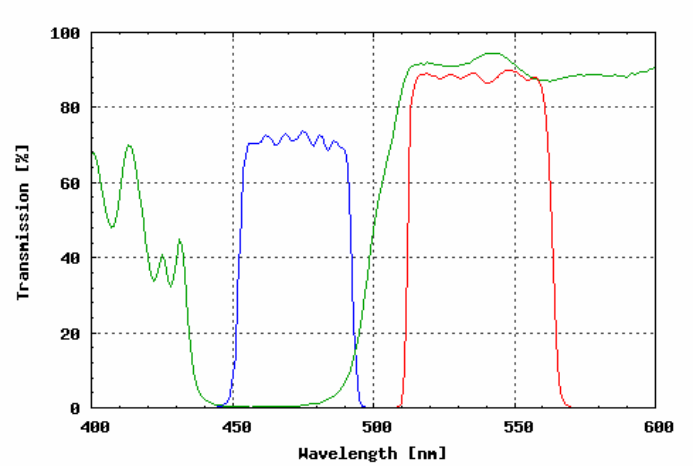
Specifications:

Instrument Specifications

Weight: 25 lbs (11kg)
Dimensions: 6'' x 8.5'' x 14'' (15cmx22cmx36cm)
Wall Adaptor: 100-240 AC, 50-60 Hz, 0.8 A
Power to instrument : 12 V DC, 2.25 A Max

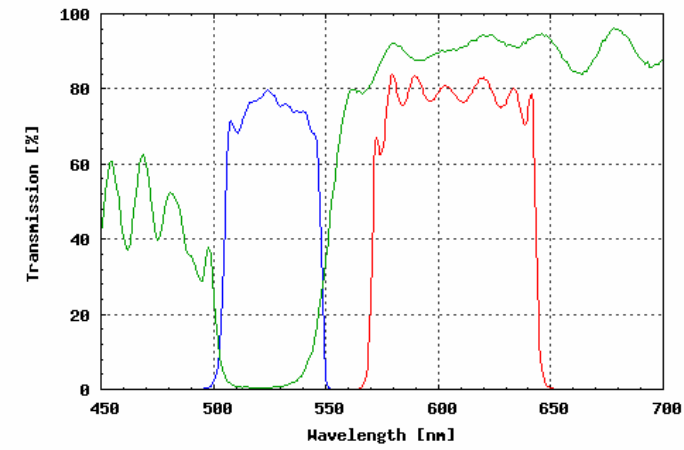
Fluorescence Specifications:

Filter set 1



Blue LED Excitation: 470nm
FITC/AlexaFluor® 488 Excitation: 495nm; Emission 519nm
Acridine Orange (+DNA) Excitation: 500nm; Emission 526nm

Filter Set 2



Green LED Excitation: 525
Propidium Iodide (PI) Excitation: 536; Emission: 617

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Getting Support

Technical Support:

Nexcelom Technical Support is available to assist with any technical issues or questions you might have during the installation or operation of the Cellometer Vision:

Email: support@nexcelom.com
Phone: +1-978-327-5340
Fax: +1-978-327-5341

Technical Support is available: Monday – Friday 8:00 AM to 5:00 PM Eastern US Time.

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Warranty Information

Warranty Information

Nexcelom warrants that Nexcelom instrumentation products shall, for a period of 12 (twelve) months from the date of purchase, be free of any defect in material and workmanship. The sole obligation of this warranty shall be to either repair or replace at our expense the product, at manufacturers option. The original sales receipt must be supplied for warranty repair. Products, which have been subjected to abuse, misuse, vandalism, accident, alteration, neglect, unauthorized repair or improper installation, will not be covered by warranty.

Any Product being returned is to be properly disinfected and packaged (in original packing if possible). Damage sustained in shipping due to improper packing will not be covered by warranty.

A valid Return Material Authorization Number (RMA#) is required for all warranty repairs. For RMA instructions, please contact our customer service department at 978-327-5340 or email support@nexcelom.com.

License Agreement

This agreement states the terms and conditions upon which Nexcelom Bioscience LLC (Nexcelom) offers to license to you the software together with all related documentations. The Software is licensed to you for use only in conjunction with Nexcelom's family of products.

Limitation of Liability (Hardware and Software)

Cellometer® branded automatic cell counting instruments, software and consumables are intended for research use only.

In no event shall Nexcelom be liable for any damages whatsoever (including, without limitation, incidental, direct, indirect, special or consequential damages, damages for loss of business profits, business interruption, loss of business information) arising out of the use or inability to use this Software, Consumables or related Hardware.

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